Intermediate filament expression in human neuro-ectodermal brain tumors

INTERMEDIATE FILAMENT EXPRESSION IN HUMAN NEURO-ECTODERMAL BRAIN TUMORS

INTERMEDIATE FILAMENT EXPRESSION IN HUMAN NEURO-ECTODERMAL BRAIN TUMORS

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE GENEESKUNDE
AAN DE RIJKSUNIVERSITEIT LIMBURG TE MAASTRICHT,
OP GEZAG VAN DE RECTOR MAGNIFICUS, PROF. DR. F.I.M. BONKE,
VOLGENS HET BESLUIT VAN HET COLLEGE VAN DEKANEN
IN HET OPENBAAR TE VERDEDIGEN OP

VRIJDAG 18 APRIL 1986, DES NAMIDDAGS OM 2.00 UUR

IN HET HOOFDGEBOUW VAN DE RIJKSUNIVERSITEIT LIMBURG,

DOOR

MARCELLINUS JOHANNES HUBERTUS MARIA HERPERS
GEBOREN TE HEERLEN IN 1957.

1986
Druk: Schrijen-Lippertz bv, Voerendaal

PROMOTORES: PROF. DR. F.T. BOSMAN
RIJKSUNIVERSITEIT LIMBURG
PROF. DR. J.L. SLOOFF
KATHOLIEKE UNIVERSITEIT NIJMEGEN

REFERENTEN: UNIV.-DOZ. DR. H. BUDKA
UNIVERSITÄT WIEN
PROF. DR. J. DRUKKER
RIJKSUNIVERSITEIT LIMBURG
DR. F.C.S. RAMAEKERS
KATHOLIEKE UNIVERSITEIT NIJMEGEN

Der geist der Medizin ist leicht zu fassen!
Ihr durchstudiert die gross und kleine Welt,
Um es am Ende gehn zu lassen,
Wie's Gott gefällt.

Mephistopheles, In Faust I,
J.W. von Goethe.

Voorwoord

Het voorliggend proefschrift kwam voor het grootste deel tot stand binnen het Neurologische Instituut van de Universiteit van Wenen (Obersteiner Insituut) te Oostenrijk (Hoofd: Prof. Dr. F. Seitelberger). De afronding volgde binnen de pathologisch-anatomische afdeling van de Katholieke Universiteit Nijmegen (Hoofd: Prof. Dr. G.P. Vooys).
Velen hebben bewust of soms onbewust bijgedragen tot de ontginning en cultivering van mijn wetenschappelijke mogelijkheden.
Allereerst dank ik U Hooggeleerde Mol, voor de ruimte die U mij in ons gezamenlijke neurophysiologische onderzoek gaf, om mijn ideeën en theorieën zonder enige schroom op voet van gelijkheid ter discussie te stellen. Het werd de basis voor mijn wetenschappelijke geaardheid.
Vielen Dank gebühre ich Ihnen, Herr Professor Seitelberger, denn es war Ihre persönliche Einladung, die mich veranlasste, während meiner Studienzeit zweimal im Rahmen eines "Wahl-Praktikum" meine Kenntnisse der Neuropathologie im allgemeinen und die der Hirntumordiagnostik im insbesondere, zu vertiefen. Es wurden Wochen die nach meinen Studienabschluss fast notgedrungen zu anderthalb nicht nur wissenschaftlich sehr wertvollen Jahren in Wien führten.
Auch Ihnen, Herr Dozent Budka, möchte ich an dieser Stelle herzlich danken für Ihre wissenschaftliche Führung und für die Zusammenarbeit.
Ihre äusserst kritische und prägnante Denkart wird mir auch weiterhin immer ein Beispiel sein.
Dir, Klaus Kitz, danke ich für Deine Freundschaft und Deine zeitweise so unentbehrliche Relativierung von Mensch und Wissenschaft.
Dank ook aan U Hooggeleerde Slooff. Het waren Uw bemoeienissen die mij in staat stelden mijn onderzoekingen in Nederland voort te zetten. Van Uw ervaring en steun bij de vaak zo moeilijke hersentumordiagnostiek heb ik dankbaar gebruik gemaakt.
Frans Ramaekers, hartelijk dank voor de vriendschappelijke en waardevolle samenwerking. Jouw deskundigheid in de celbiologie vormde een verrijking van mijn neuropathologisch denken.
U, Hooggeleerde Bosman, ben ik zeer erkentelijk voor al Uw constructieve kritische kanttekeningen en Uw persoonlijk engagement bij de afronding van dit werkstuk. De discussies met U werkten zeer verhelderend.
Zeer hartelijk dank ik ook mijn huidige opleiders in de Neurochirurgie: H. v. Dulken, Dr. R.T.W.M. Thomeer en J.H.C. Voormolen, voor hun interesse in mijn onderzoekingen en voor de ruimte die zij creëerden om naast de klinische werkzaamheden, mijn proefschrift te kunnen voltooien.
Zonder de nimmer aflatende energie, het geduld en de volharding waarmee Mej. Ouwehand aan het manuscript gewerkt heeft, zou alles eerst maanden later voltooid zijn geweest. Ik ben je hiervoor zeer dankbaar Wilma.
Slechts met jouw steun is het mogelijk geweest Rina. Het is daarom mede jouw proefschrift.

Leiden, 15 februari 1986.

Contents

Chapter 1

Chapter 2

Classification of Central nervous system tumors

Chapter 3

Intermediate filament protein immunohistochemistry in the histopathology of brain tumors

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Chapter 1

1.1 General introduction

Since the initial isolation and characterization of the glial fibrillary acidic protein (GFAP) from multiple sclerose plaques in the early seventies (Eng et al., 1971) considerable evidence has accumulated supporting the view that GFAP is the major protein constituent of glial filaments (Eng and DeArmond, 1982). Its reliable detection by immuno-cytochemical methods has facilitated the study of glial cells in the normal, and pathologically altered central nervous system (CNS), as well as in CNS neoplasms, both in vivo and in vitro.
The glial filaments, of which GFAP is the main protein constituent, are highly insoluble structures of intermediate-sized filament (IF)-type. IF occur in virtually all vertebrate cells and form a part of the cytoskeleton. Four other major classes of IF proteins have been recognized, which have been shown to occur in a tissue or cell specific manner (Franke et al, 1981; Osborn et al, 1981). Thus, cytokeratin filaments are found in virtually all epithelial cells, while vimentin filaments occur in various non-epithelial cells, especially those of mesenchymal origin. Desmin filaments have been found in various types of muscle cells and the neurofilament proteins have been detected almost exclusively in neuronal cells.
Tumors derived from these different types of tissues in most cases retain their specific IF protein(s) (Osborn and Weber, 1983; Ramaekers et al, 1983). In astrocytomas for exemple GFAP has been consistently found in the neoplastic astrocytes (Bignami et al, 1980; Eng and DeArmond, 1982).
In immature glia and fibrous astrocytes as well as in cultured glial cells (Quinlan and Franke, 1983) both GFAP and vimentin are synthesized. Apparently, the expression of IF protein in glial cells varies according to the state of differentiation and the activity of proliferation. It has been postulated that differentiational characteristics of neoplastic cells often reflect the various stages in the differentiation and maturation of their normal counterparts (Rubinstein, 1985). According to this hypothesis, in glial neoplasms GFAP expression, but potentially also vimentin expression might be expected.
Since both GFAP and vimentin can be easily detected immunohistochemically, alterations in the IF component of neoplastic neuro-ectodermal cells can be reliably studied by light microscopy.

1.2 Aim of this study

The aim of our studies was to document the expression of IF proteins in neuro-epithelial central nervous system tumors. Potentially the results might improve our understanding of patterns of differentiation in these neoplasms and lead to a better classification of these neoplasms which are often rather difficult to classify.

The hypothesis formulated above leads to the following specific questions.

1. Is the expression of GFAP in neoplastic astrocytes related to the grade of malignancy of the neoplasm?
2. Is vimentin expressed by neoplastic astrocytes and if so is its degree of expression related to the grade of malignancy of the neoplasm?
3. Is expression of GFAP and vimentin in neoplastic astrocytes subject to microenvironmental influences?
4. Is GFAP expressed in neoplastic oligodendrocytes and if so, is the expression related to a mixed oligo-astrocytoma pattern of differentiation?
5. How frequently is GFAP expressed in primitive neuro-ectodermal tumors, especially in the so-called circumscribed cerebellar arachnoidal sarcoma and what is the biological significance of this phenomenon?
6. Are GFAP and vimentin colocalized in malignant astrocytes or are they located in different cellular domains?
7. Can the findings be applied to neuropathology, especially for the diagnosis of malignant tumors or for the differentiation of benign and malignant astrocytes?

These questions will be addressed in the following chapters.

1.3 References

1. **Bignami, A., Dahl, D., Rueger, C. (1980):**
   Glial fibrillary acidic (GFA) protein in pathological conditions. Vol. 1, p 285-319.
   S. Fedoroff and L. Hertz, eds., Academic Press, New York, London.
2. **Eng, L.F., Vanderhaegen, J.J., Bignami, A., Gerstl, B. (1971):**
   An acidic protein isolated from fibrous astrocytes.
   Brain Res. 28: 351-354.
3. **Eng, L.F., DeArmond, S.J. (1982):**
   Immunochemical studies of astrocytes in normal development and disease.
   Adv. Cell. Neurobiol. 3: 145-171.
4. **Franke, W.W., Schmidt, E., Schiller, D.L., Winter, S., Jarash, E.D., Moll, R., Denke, H., Jackson, B.W., Illmensee, K. (1981):**
   Differentiation-related patterns of expression of proteins of intermediate-sized filaments in tissues and cultured cells.
   Cold Spring Harbor Symp. Quant. Biol. 46: 431-453.
5. **Osborn, M., Geisler, N., Shaw, G., Sharp, G., Weber, K. (1981):**
   Intermediate filaments.
   Cold Spring Harbor Symp. Quant. Biol. 46: 413-429.
6. **Osborn, M., Weber, K. (1983):**
   Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology.
   Lab. Invest. 48: 372-394.
7. **Quinlan, R.A., Franke, W.W. (1983):**
   Molecular interactions in intermediate filaments revealed by chemical cross-linking. Heteropolymers of vimentin and glial filament protein in cultured human glioma cells.
   Eur. J. Biochem. 132: 477-484.
8. **Ramaekers, F.C.S., Puts, J.J.G., Moesker, O., Kant, A., Huysmans, A., Haag, D., Jap, P.H.K., Herman, C., Vooys, G.P. (1983):**
   Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumors; an overview.
   Histochem. J. 15: 691-713.
9. **Rubinstein, L.J. (1985):**
   Embryonal central neuroepithelial tumors and their differentiating potential.
   J. Neurosurg. 62:795-805.

Chapter 2

Classification of Central Nervous System Tumors

2.1 Classification

Modern classifications of central nervous system (CNS) tumors are mostly based upon the concept of embryogenetic derivation as embodied in the classification of Bailey and Cushing (1926).
The classification proposed by these investigators is developmental, since it attempts to classify brain tumors according to the type and developmental stage of normal brain cells, with which the tumor cells correspond morphologically.
An alternative approach, which is frequently encountered in oncological pathology is the histogenetic classification, in which tumors are classified according to the "tissue" from which they probably are derived.
Recent electron microscopic investigations and immunohistochemical studies, including our own, support the developmental concept for at least some of these brain tumors, as will be discussed later on.
Historically, four classification concepts have been used by the anglo-american, the german and the spanish latin american neuropathological schools.

A. The classification of Bailey and Cushing (1926)
In their first monograph these authors distinguished 14 tumor types which were subdifferentiated into 20 cell types, and all derived from medullary epithelium. Subsequently Bailey (1927) excluded a group of tumors which according to modern concepts would be called primitive neuro-ectodermal and changed the originally used term "spongioblastoma multiforme" into "glioblastoma multiforme". Bergstrand (1932 and 1937) added to this concept a separation of cerebellar astrocytomas from cerebral astrocytomas and described the differences between benign and malignant gliomas. Russell and Rubinstein (1963) proposed the following classification of tumors of glial origin:

1. astrocytic group:
   a. astrocytoma
   b. astroblastoma
   c. polar spongioblastoma
2. oligodendroglioma
3. tumor of the ependyma and its homologies:
   a. ependymoma
   b. subependymoma
   c. choroid plexus papilloma,
      colloid cysts

4. glioblastoma multiforme
With only one minor change, (the separation of the choroid plexus papilloma tumors from those derived from the ependyma) this is the widely used standard-classification of Rubinstein (1972).

B. The classification of Del Rio Hortega (1932)
The classification of Del Rio Hortega is widely used in South America. It is also based on a developmental concept and is therefore nearly identical to the classification of Bailey and Cushing, except for its subdivision of paragliomas. However the terminology and definiton of certain tumor types (i.e. the astroblastoma and spongioblastoma) is different.

C. The classification of Kernohan et al (1949)
Kernohan et al (1949) introduced a system of grading in the field of brain tumors, which was modified by Kernohan and Sayre in 1952.
It was based on the concept of a continuous series of gradations with increasing malignancy from fibrillary and protoplasmatic astrocytomas to glioblastomas. A comparable series of stages for other oligodendrogliomas was merely indicated, since an exact definition of the four grades of malignancy was given only for the astrocytoma and ependymoma tumor group. Four main groups of neuro-ectodermal tumors were postulated:
a. astrocytomas
b. ependymomas
c. oligodendrogliomas
d. neuroastrocytomas
Ringertz (1950) almost at the same time described a grading system with only three grades of malignancy and restricted his system to the astrocytomas, oligodendrogliomas and ependymomas.
These different classification systems have been critized rather frequently, mainly in relation to the confusion which occured due to the variable nomenclature of various tumors, i.e. spongioblastoma, astroblastoma and ependymoblastoma, depending on different pathogenetic concepts by neuropathologists from various "neuropathological schools" in different countries. In 1961 Zülch tried to overcome this problem, by working out an acceptable compromise between the existing classifications, facilitating the adoption of a more uniform terminology of brain neoplasms. The principles of this suggestion were published by the Unio Internationalis Contra Cancrum (UICC) in an Atlas of Human Neoplasms (1965).

D. The WHO-classification
In 1979 a World Health Organisation (WHO)-classification was published. It contained a proposal prepared by Zülch back in 1970, and was in fact a compromise between the "Zülch-school" and the nomenclature used by the "anglo-american school" represented by Rubinstein. The history and the concept of the WHO-classification have been described extensively by Zülch (1978).

2.2 The classification used for the neuro-ectodermal brain tumors in the present studies essentially consisted of the WHO-classification by Zülch (1979), however with an extension concerning the incorporation of medulloblastomas and

neuroblastomas into the group of primitive neuroectodermal tumors (PNET) (Hart and Earle, 1973). This concept has been supported by Rorke (1983), based on lightmicroscopic-, electronmicroscopic-, and immunohistochemical characteristics. Grading was performed according to the concepts of Kernohan (1949) and Kernohan and Sayre (1952) with regard to the gliomas.

Classification of CNS tumors used in the present study:
Neuroepithelial tumors:

I. Astrocytic:
   1. Astrocytoma:
      a. fibrillar
      b. protoplasmatic
      c. gemistocytic
      d. mixed
      e. others
   2. Pilocytic astrocytoma
   3. Subependymal giant-cell astrocytoma
   4. Astroblastoma
   5. Meningocerebral pleomorphic xanthoastrocytoma
   6. Anaplastic astrocytoma

II. Oligodendroglial and mixed oligoastrocytic:
   1. Oligodendroglioma
   2. Anaplastic oligodendroglioma
   3. Oligoastrocytoma
   4. Anaplastic oligoastrocytoma

III. Ependymal:
   1. Ependymoma:
      a. classic
      b. myxopapillary
      c. papillary
      d. foramen-Monroi-type
      e. subependymoma
      f. others.
   2. Anaplastic ependymoma
   (3. Ependymoblastoma see later VII 2b).

IV. Derivatives from the plexus chorioideus:
   1. Plexus papilloma
   2. Malignant plexus papilloma (plexuscarcinoma)

V. Derivatives from the pineal parenchyma:
   1. Pinealocytoma (Pineocytoma)
   (2. Pinealoblastoma see later VII 2)

IV. Neuronal and mixed neuronal-glial:
   1. Gangliocytoma
   2. Anaplastic gangliocytoma
   3. Ganglioglioma
   4. Anaplastic ganglioglioma
   (5. Neuroblastoma and ganglioneuroblastoma see later VII 2b)

VI. Glioblastomal:
    1. Glioblastoma:
        a. multiforme
        b. giant-cell glioblastoma
        c. glioblastoma with sarcomatous components (gliosarcoma)
        d. others
VII. Embryonal:
    1. Medulloepithelioma
    2. Primitive Neuro-Ectodermal Tumor (PNET) and Medulloblastoma (cerebellar PNET) and Pinealoblastoma (pineal parenchymal PNET) subdefined "desmoplastic" with a prominent mesenchymal component (i.e. desmoplastic medulloblastoma)
        a. without differentiation characteristics
        b. with differentiation characteristics
            i. glial (astrocytic and/or oligodendroglial)
            ii. ependymal (ependymoblastoma).
            iii. neuronal (Neuroblastoma and ganglioneuroblastoma).
            iv. bi-or pluripotential
VIII. Others.

2.3 Neuroepithelial tumors, a brief description

2.3.1 Introduction

According to Rubinstein (1972) primary tumors of the central nervous system and the meninges account for approximately 9% of all primary neoplasms, of which 85% are found in the cranial cavity.
About half of this 85% consists of the glioma-group. This group includes not only those tumors which are derived from neuroglial-, but also those from neuronal-cells and their primitive bi/multi-potential precursors. By *morphological criteria, gliomas often contain anaplastic tumor cell populations.* Gliogenous tumors are presently classified according to the three types of adult glial cells normally found in the human central nervous system: the astrocyte, the oligodendrocyte and the ependymal cell. The medulloblastoma is considered a derivative from primitive embryonal cells, possibly differentiating along more than one cell line, and was only recently grouped with other primitive neuro-ectodermal tumors by Rorke (1983), as mentioned earlier. Extensive histological definitions or a description of characteristics of these various neuro-ectodermal tumor entities are not provided here because excellent descriptions are avaliable (Rubinstein, 1972; Zülch, 1956 and 1979).

2.3.2 Astrocytoma
(astrocytoma grade I and II).

Astrocytomas are usually slowly growing neuro-ectodermal neoplasms derived from and consisting of astrocytes showing various degrees of maturity and/or differentiation.
This group includes the protoplasmic, fibrillary, piloid or pilocytic and

gemistocytic astrocytoma, which probably reflect the different structural forms of normal and reactive astrocytes. These tumors show some differences in their gross features, microscopic appearances and biological behavior. The different cell forms often occur together in the same neoplasm.
Another way of separating this tumor group into well defined clinicopathological entities is provided by the localisation: each area is generally associated with a prevalent age incidence, a characteristic microscopic picture, and a fairly predictable biological behavior. For example the astrocytoma in the cerebral hemisphere is most frequently found in adults in the third and fourth decades. In contrast, those located in the hypothalamic region or cerebellum and pons are most often seen in children and adolescents. The (primary) clinical presentation of these neoplasms depends largely on their localization in the human brain or spinal cord, which characteristically can convert even the smallest histological benign space occupying lesion into a clinical malignant one.
Despite their histologically benign appearance and their slow growth rate, many astrocytomas undergo dedifferentiation within an undefined period, terminating with the histologic picture of glioblastoma multiforme.
The diffuse cerebral astrocytomas are microscopically characterized by a diffuse infiltration of the grey and/or white matter. The cellular density is sometimes only slightly higher than that of the normal brain. With silver carbonate preparations the cellular outlines of tumor cells appear to be larger, the cells showing more processes than normal astrocytes. The nuclei are usually also slightly larger and more irregular than in normal astrocytes, with mild hyperchromasia.

2.3.3 Malignant astrocytoma

(Astrocytoma grade III or dedifferentiated astrocytoma).

Microscopically these tumors show an increased cellularity of astrocytic lineage, nuclear irregularity and hyperchromasia, vascular endothelial proliferation, and a variable number of mitotic figures. This illustrates that the differential diagnosis between astrocytoma grade III and glioblastoma multiforme is often "an academic exercise" especially if one considers the similarity in biological behavior of these histological tumor-entities.

2.3.4 Glioblastoma multiforme

(glioblastoma; spongioblastoma multiforme, astrocytomas grade IV).

In the older age groups the glioblastoma is the most anaplastic form of primary intracranial CNS-neoplasm, and nowadays widely accepted as an extreme manifestation of anaplasia of mature astrocytic glial cells. However, some authors believe that the astrocytic derivation is not really evident. Scherer (1940) proposes that there exist two types of glioblastoma: a primary which arises "de novo" , and a secondary type which is the result of dedifferentiation in an astrocytoma. Rubinstein (1972), however, concludes that most glioblastomas are probably derived by anaplastic changes in a preexisting astrocytoma.
This rapidly growing tumor may arise in any part of the CNS and usually causes symptoms in less than six months prior to the diagnosis. The peak incidence is between 45 and 55 years of age.
Histologically this tumor shows a high intra- and intertumor variability. There

are more or less densely cellular areas composed of neoplastic astrocytes of varying size and shape, showing considerable nucleocytoplasmic abnormalities, and variable numbers of partially atypical mitotic figures. Characteristic are:

a. necrotic zones, around which the tumor cells are arranged in "pseudopalissades"
b. hemorrhagic zones.
c. cysts and
d. a striking vascular endothelial proliferation, with numerous highly convuluted capillary blood vessels, with evidence of active proliferations of endothelial cells.

Sometimes, the endothelial and/or perivascular fibroblastic proliferation is so abundant, simulating neoplastia, resultating in a picture of mixed glioma and fibrosarcoma, sometimes falsely called "gliosarcoma". Clinically and biologically these "gliosarcomas" essentially behave like glioblastomas.
Bizarre, irregular giant- and multinucleated cell forms are frequently found in glioblastoma, and sometimes dominate the histological picture in such a manner that some authors regard them as a separate group. Like the glioblastomas these "giant-cell glioblastomas" often contain a considerable connective tissue proliferation, erroneously leading to diagnoses of giant-cell, or monstro-cellular sarcomas, since they are merely a variant of the glioblastomas.

2.3.5 Oligodendroglioma

(oligodendrocytoma, oligodendroblastoma).

This tumor, which contains oligodendrocytes in various levels of differentiation is normally found in the cerebral hemispheres, usually invading the cortex and the white matter, with a peak incidence between the ages of 30 and 50.
It is difficult to define the oligodendroglioma as a clinocopathologic entity since this tumor-group shows a considerable variation in its natural history, and its biological behavior. Except for the series of Smith et al. (1983) no valid correlation has been established between the histologic features and the clinical evolution of oligodendrogliomas.
Microscopically, the tumor shows a characteristic and highly uniform appearance consisting of swollen, closely packed oligodendrocytes with a small, round, darkly staining nucleus surrounded by a clear halo. The tumor shows a regular delicate vascular stroma which intersects the uniform sheets of tumor cells and intensifies the characteristic "honeycomb" aspect.
Apart from this characteristic microscopical picture, a great deal of oligodendrogliomas present atypical histologic appearances, with f.e. a less defined vascular stroma, or containing atypical cells without a clear halo, sometimes perivascularly located, immitating an ependymoma.

The incidence of mitotic figures is highly variable. Foci of calcification and hemorrhage are often seen. Frequently these tumors grow slowly and may show a long evolution. Others grow rapidly and may undergo malignant change clinically and histologically into a glioblastoma multiforme.

2.3.6 Mixed oligo-astrocytoma.

It is not uncommon for oligodendrogliomas to contain astroglial elements,

closely intermingled with the neoplastic oligodendrocytes, or in distinct foci of pure astrocytoma. The presence of these mixed neoplastic populations causes important cytologic problems in predicting the further evolution of the tumor, and the expression of its malignant potential. According to Rubinstein (1972) probably 50% of oligodendrogliomas in fact contain such mixed cell forms. Nevertheless the biological behavior of these tumors usually seems to be that of an oligodendroglioma.

2.3.7 Medulloblastoma

Medulloblastomas are generally considered as embryonic tumors and account for approximately 6% of all intracranial tumors of the glioma group. In children they comprise about one-third of the tumors in the midline of the posterior fossa. More than 50% of the cases occur in the second half of the first decade. However nearly 30% of the cases occurs in adolescence and early adulthood, and these are usually situated in a more lateral position at the cerebellar lobes (Rubinstein, 1972).
Since the introduction of the concept of the cerebellar medulloblastoma by Bailey and Cushing (1925), the problem of histogenesis and differentiation potencies of this tumor has been subject to speculation and discussion by many authors. One commonly accepted theory is that the medulloblastoma is derived from remnants of the fetal external granular layer which normally persists in the infant cerebellum until 12 months post partum (Stevenson and Ecklin, 1934). Another concept has recently been presented by Rorke (1983) in which the medulloblastoma, together with other primitive neuro-ectodermal tumors (PNET's) (ependymoblastoma, pinealoblastoma, neuroblastoma and ganglioneuroblastoma, primitive polar spongioblastoma), are thought to be the result of neoplastic transformation of primitive neuroepithelial cells in persistent subependymal zones at all levels of the CNS or pineal body.
Clinically the medulloblastomas always present as posterior fossa tumors. Microscopically the tumors are densely cellular with cells showing hyperchromatic nuclei and very scant or absent cytoplasmic outlines. The cells are arranged in dense sheets or (in about one third of the cases) "Homer Wright rosettes" (Wright, 1910), called "pseudo-rosettes" by Bailey and Cushing, (1926), which are highly characteristic. Arrangement of the nuclei in a rhythmic pattern can also be encountered, as well as the formation of "pseudopallissades" around areas of necrosis.
Jänish et al (1976) distinguish histologically between a classical form of medulloblastoma (Bailey and Cushing, 1925) and small- and large- cell medulloblastomas. This, however, does not seem to be of any practical importance since no differences in clinical or biological behavior are mentioned in their report.

The desmoplastic medulloblastoma (Rubinstein and Northfield, 1964) or the so-called "circumscribed arachnoidal cerebellar sarcoma" (Foerster and Gagel, 1938) is a variant which is subject to controversy and object of our own studies. This medulloblastoma variant, is usually located partly extracerebellar, and a characteristic histologic picture is encountered using reticulin-stains.
It shows an arrangement of sinuous trabeculae and pale islands of

medulloblastoma tumor cells entirely devoid of fibrous connective tissue, delineated from the surrounding reticulin rich tumor parts by a limiting reticulin membrane.
Rubinstein and Northfield (1964) regard these tumors as a variant of medulloblastoma in which the above described histological characteristics are a result of leptomeningeal invasion with an extensive connective tissue reaction. Foerster and Gagel (1938) on the other hand distinguish arachnoidal cerebellar sarcoma from the classical medulloblastoma group of the midline, not only on histological grounds, but also because of differences in localization, age incidence, and prognosis, which is much better with the arachnoidal sarcoma.

2.4 References

1. **Bailey, P. (1927):**
   Histological atlas of gliomas.
   Arch. Pathol. 4:871-921.
2. **Bailey, P. and Cushing, H. (1925):**
   Medulloblastoma cerebelli, common type of mid-cerebellar glioma of childhood.
   Arch. Neurol. Psychiat. 14:192-224.
3. **Bailey, P. and Cushing, H. (1926):**
   Tumors of the glioma group.
   J.B. Lippincott, Philadelphia.
4. **Bergstrand, H. (1932):**
   Ueber das sogenannte Astrozytom des Kleinhirn.
   Virchows Arch. path. Anat. 287:538.
5. **Bergstrand, H. (1932):**
   On gliomas of the cerebral hemispheres.
   Acta path. microbiol. scand. 11:100.
6. **Bergstrand, H. (1937):**
   Weiteres über sogenannte Kleinhirnastrozytome.
   Virchows Arch. path. Anat. 299:725-739.
7. **Foerster, O., Gagel, O. (1938):**
   Das umschriebene Arachnoidalsarkom des Kleinhirns.
   Z.Ges.Neurol. Psych. 164:565.
8. **Hart, M.N. and Earle, K.M. (1973):**
   Primitive neuro-ectodermal tumors of the brain in children.
   Cancer 32:890-897.
9. **Hortega, P. Del Rio (1932):**
   Estructura y systematisa cion de los gliomas y paragliomas.
   Arch. espan. Oncol. 2:411-677 (1932).
10. **Jänish, W., Güthert, H. and Schreiber, D. (1976):**
    In: Pathologie der Tumoren des Zentralnervensystem.
    VEB Gustav Fischer Verlag, Jena p. 177-253.
11. **Kernohan, J.W., Mabon, R.F., Svien, H.J., Adson, W. (1949):**
    Simplified classification of gliomas.
    Proc. Mayo. Clin. 24:71-75.
12. **Kernohan, J.W. and Sayre, G.P. (1952):**
    Tumors of the central nervous system.
    Armed Forces Institute of Pathology, Washington D.C.
13. **Ringertz, U. (1950):**
    Grading of gliomas.
    Acta path. scan. (Copenh.) 27:51-64.
14. **Rorke, L.B. (1983):**
    The cerebellar medulloblastoma and its relationship to primitive neuro-ectodermal tumors.
    J. Neuropathol. Exp. Neurol. 42:1-15.

15. **Rubinstein, L.J. (1972):**
Tumors of the central nervous system.
In: Atlas of tumorpathology, Series 2. Fascicle 6 Washington D.C.: Armed Forces Institute of pathology.
16. **Rubinstein L.J., Northfield, D.W.C. (1964):**
The medulloblastoma and the so-called "arachnoidal cerebellar sarcoma". A critical reëxamination of a nosological problem.
Brain 87:379-412.
17. **Russell, D.S. and Rubinstein, L.J. (1963):**
Pathology of tumours of the nervous system; second edition, E. Arnold, London.
18. **Scherer, H.J. (1940):**
Cerebral astrocytomas and their derivatives.
Amer. J. Cancer 40:159-198.
19. **Smith, M.T., Ludwig, C.L., Godfrey, A.D., Armbrustmacher, V.W. (1983):**
Grading of Oligodendrogliomas.
Cancer 52: 2107-2114.
20. **Stevenson, L. and Ecklin, F. (1934):**
Nature and origin of some tumors of the cerebellum-medulloblastoma.
Arch. Neurol. Psychiat. 31:93-109.
21. **Wright, J.H. (1910):**
Neurocytoma or neuroblastoma, a kind of tumor not generally recognized.
J. Exp. Med. 12:556-561.
22. **Zülch, K.J. (1956):**
Biologie und Pathologie der Hirngeschwülste.
In: Hdb. Neurochir., Hrsg. Olivecrona, H., Tönnis, W., Bd. 3, 1-702. Berlin: Springer.
23. **Zülch, K.J. (1978):**
Principles of the new WHO classification of brain tumors.
J. Neurosurg. Sci. 22:1-5.
24. **Zülch, K.J. (1979):**
Histological typing of tumors of the central nervous system. International Histological Classification of Tumors. No 21, WHO, Geneva.

Chapter 3

Intermediate filament protein immunohistochemistry in the histopathology of brain tumors.

3.1 IF proteins as cytoskeletal constituents

In recent years evidence has accumulated that the cytoarchitectural basis of most nucleated cells consists, amongst others, of a system of three different types of filaments. By electronmicroscopy microfilaments, microtubules and intermediate filaments can be distinguished.
These three fibrillar matrices together form the cytoskeleton. Extensive biochemical, immunochemical and morphological studies have shown that the structure of these filaments and their dynamic state determine the shape and motile properties of cells.

Microfilaments are thin filaments with an average diameter of 4-6 nm occurring in larger or smaller bundles(stress-fibers), or as a diffuse network located at the cell periphery. Condeelis (1981) proposed that this microfilamentous network might be responsible for the gellike consistency of the marginal cytoplasm which is important in retaining the cell-shape or, on the other hand, might be involved in cell-movement.The core protein of micro-filaments is actin, of which several isoforms are known and which associates with several other (contractile) proteins.

Microtubules are hollow structures with an exterior diameter of 25 nm and varying length. They are involved in various types of cell movement, i.e. phagocytosis, movement of secretory granules and other organelles, as well as in mitosis. The protein constituents of microtubules are α- and β- tubulin.

Intermediate filaments are fibrous structures with a diameter of 7-11 nm, which under physiological conditions consist of highly insoluble protein chains. Intermediate filaments have a tendency to associate with other cellular structures, such as microtubules, nuclear and cellular membranes (Ramaekers et al. 1982), and other specific proteins (Lazarides, 1980; Anderton, 1981). In relation to the subject of this thesis, intermediate filaments will be more extensively reviewed.

3.2.1 Intermediate filament proteins (IFP) as tissue specific markers

Five major classes of IF constituting proteins have been identified (Franke et al., 1981; Osborn et al., 1981):

IFP	MW (kD)	Occurrence
Vimentin	57	mesenchymal cells
Desmin	53-56	muscle cells
Cytokeratins	40-68*	epithelia
Glial fibrillary acidic protein (GFAP)	49	astrocytes
Neurofilament proteins	68, 150, 200	neuronal cells

* 19 different polypeptides

Several investigators have produced antibodies, monoclonal as well as polyclonal, against individual constituent proteins and have shown that although the different types of IFP share antigenic determinants (Pruss et al. 1981), unique antigenic domains exist, that allow the production of antibodies reacting specifically with one type of IFP. The use of such specific antibodies has shown that IFP are distributed in a tissue specific manner.

In general, tumor cells retain the IFP of the cell type from which they are derived (Gabbiani et al., 1981; Osborn and Weber, 1983; Ramaekers et al., 1983a). Although exceptions to this rule have been reported (Gould, 1985), IFP may serve as differentiation markers for the identification of cells or tumors of unknown origin.

The IFP which may occur in brain tumors will be discussed in some more detail.

3.2.2 Vimentin.

Vimentin is the IFP of cells of mesenchymal origin (e.g. fibroblasts and endothelial cells), Franke et al. (1978). However, in most cultured cells vimentin can be demonstrated, irrespective of the cell type from which the cultures were derived (Bennett et al. 1978; Franke et al., 1979). Also several types of tumors coexpress vimentin in addition to their tissue specific IFP. Furthermore cells in the developing embryo may transiently express this protein.
In the developing and mature nervous system in the mouse, vimentin intermediate filaments occur in astrocytes in a considerable range of developmental stages, even before the onset of GFAP expression (Schnitzer et al., 1981). Therefore vimentin might serve as a marker of immature glial cells. Vimentin has been found in cultured glial cells, in immature glia, in normal and reactive astrocytes (Dahl et al., 1981a and 1981b), and in coexpression with GFAP in the same cell (Schnitzer et al., 1981; Dahl et al., 1981c). The coexpression of these proteins in neoplastic glial cells has been explicitly studied only by Roessmann et al. (1983) and

Yung et al. (1985), which showed that astrocytomas contain vimentin- in addition to GFAP-positive tumor cells. Part of this thesis deals with this coexpression.

3.2.3 Cytokeratins

Cytokeratins are the protein constituents of the epithelial type of IF. These cytokeratin filaments are characterized by a remarkable biochemical diversity, represented in human tissues by at least 19 different cytokeratin polypeptides (Moll et al., 1982). These polypeptides are not expressed randomly throughout epithelia but occur in cell-type specific combinations. Thus, antibodies to cytokeratins do not only recognize keratinizing epithelia, but also immunochemically react with IF in more "simple" epithelial tissues, such as glandular and columnar epithelial tissues, transitional epithelium, mesothelium and myoepithelium.
Since cytokeratins are generally retained in primary as well as in metastatic tumors derived from epithelial tissues (Moll et al., 1982) the presence or absence of specific cytokeratin polypeptides in certain tumors can be used to sustain differential diagnosis.

3.2.4 Glial fibrillary acidic protein (GFAP)

GFAP was first isolated from multiple sclerose plaques by Eng et al. in 1971. Subsequently it has been shown that GFAP is the main protein constituent of the intermediate filament-system of glial cells. Specific anti-GFAP antisera have been used in numerous studies concerning glial cytology in normal and pathologically altered CNS as well as in CNS neoplasms (see for example Deck et al. 1978, Eng and Rubinstein 1978, DeArmond et al. 1980, Eng and DeArmond 1982).
An overview on the chemical properties, possible function, the use of GFAP in cultured human glioma cells and the expression of GFAP in astrocytes in various non-neoplastic conditions including reactive gliosis is provided by DeArmond et al. (1980) and Eng and DeArmond (1982).
Since the production by Eng and Rubinstein (1978) of a monospecific antiserum to GFAP, this protein has been used as a specific cell marker in neuro-oncology, especially in those neoplasms where classical histological stains did not allow an exact tumor diagnosis (Rubinstein, 1982).

3.2.5 Neurofilaments

The neurofilament IF proteins (68 kD, 150 kD and 200 kD) (Schlaepfer et al., 1982) occur exclusively in neuronal cells (Dahl, 1983).
The three proteins are not distributed randomly throughout neuronal tissues. All three proteins are present in large amounts in axons, whereas the 200 kD subunit is very scant in dendrites and cell bodies of pyramidal cells (Bonnin and Rubinstein, 1984).
The 200 kD protein appears later during development of the CNS than the 68 kD and the 150 kD subunits (Shaw and Weber, 1982).
Neurofilaments are biochemically and immunochemically distinct, and sera have been prepared that show no cross-reactivity with antibodies against other IFP.

Therefore, such antisera can be used as reliable markers for ganglion cells and their precursors, in the central and peripheral nervous system in health and disease.

3.2.6 The use of IFP antisera in the histopathology of brain tumors

Histopathology has shown rather drastic changes in the last decennium. Originally, histological diagnosis almost exclusively relied on purely morphological criteria. The introduction of electron microscopy and quantitative morphology (morphometry) has allowed further refinement of the morphological basis of histological diagnosis. A completely new element was introduced with the application of immunocytochemical methods in diagnostic histopathology.
Immunocytochemistry allowed biochemical analysis of tissue and cell components in situ and thus added an exciting new dimension to histopathology. Information concerning fundamental properties of cells and tissues could now be used as additional criteria for the classification of disease.

Two important developments in immunocytochemistry have greatly facilitated the acceptation of immunocytochemical methodology for diagnostic purposes. The first was the introduction of enzyme labels, which resulted in permanent preparations that could be counterstained and studied by regular absorption microscopy. Peroxidase is the most widely used enzyme label. The second development was the hybridoma technology for the production of monoclonal antibodies. This technology greatly contributed to the almost unlimited availability of a wide variety of highly specific antibodies. In the surgical pathology of neoplasia immunocytochemical detection of IFP presently plays an important role.
This is also true for neurosurgical histopathology.
Immunocytochemistry can be used to provide an answer to one or more of the following questions:

1. Is a CNS tumor primary or metastatic?
2. If it is a primary tumor, how should it be classified?
3. What is the level of differentiation of the neoplasm?
4. If it is a metastasis, what is the site of the primary tumor?

Ad. 1. IF immunohistochemistry will be used only in addition to routine light microscopy, which in the majority of the cases is sufficient to provide a correct diagnosis. Only rarely will the distinction between a primary brain tumor and a metastasis be a problem. Therefore, to solve this problem IF immunohistochemistry will be necessary in very few cases. GFAP immunoreactivity will prove an astrocytic nature of the tumor. Cytokeratin IF will indicate a carcinoma metastasis. For metastases of mesenchymal tumors (with the exception of desmin positive myogenic tumors) there may be some problems. In addition, metastases will not show any GFAP and/or neurofilament immunoreactivity.

Ad. 2. Many investigations have been published concerning the second question. Most investigations dealing with primary brain tumors describe the application of GFAP antisera since tumors of the glioma

group are the most prevalent of the primary neuroepithelial neoplasms. They show a wide range of morphological variations which often leads to diagnostic difficulties. The application of GFAP immunohistochemistry has shown to be very useful in such cases. This method allowed the distinction of astroglial tumor parts in cases of mixed oligo-astrocytomas, gangliogliomas, angiogliomas, mixed "gliosarcomas". It revealed the astrocytic origin of tumor parts invading the leptomeninges, and the identity of gliomas metastasizing to extraneural sites, or the astroglial origin of the pleomorphic xanthoastrocytoma (Kepes et al., 1979) formerly interpreted as a meningocerebral fibroxanthoma. For further details, including the GFAP immunoreactivity in ependymomas, the excellent reviews of Rubinstein (1982), Bonnin and Rubinstein (1985) and Miettinen et al. (1984) are recommended because they cover most of the literature published on this subject so far. GFAP immunoreactivity in oligodendrogliomas is reviewed in Chapter 6.
The use of antisera against neurofilaments has revealed the neuronal origin (or differentiation) of poorly differentiated primary neuroectodermal tumors (Roessmann et al., 1983) and the human esthesioneuroblastoma (Trojanowski et al., 1982). Also this area is extensively reviewed by Rubinstein (1985).

Ad. 3. Divergent differentiational capabilities of medulloblastomas, central neuroblastomas, and pineoblastomas were shown using neurofilament and GFAP immunohistochemistry (Roessmann et al., 1983). The occurrence of GFAP and/or neurofilament positive (tumor)cells in medulloblastoma has been the subject of considerable controversy. This subject is extensively reviewed in Chapter 7.
Though in the literature the degree of GFAP expression within astrocytomas is claimed to be inversely related to the grade of malignancy (Velasco et al., 1980; Marsden et al., 1983; Goebel et al., 1985) we could not confirm these findings as discussed in the Chapters 5 and 8 of this thesis.
Vimentin-IF immunohistochemistry has been used in neurooncology by only a few investigators (Osborn and Weber, 1983; Roessman et al., 1983; Borit and Yung, 1983 and Yung et al., 1985) who found vimentin positive neoplastic cells in glial tumors. Chapter 5 describes a more detailed study concerning the coexpression of GFAP and vimentin IF content by astroglial neoplasms.

Ad. 4 The use of antisera against cytokeratin-IFP (including the monoclonal determination of the different subclasses) and desmin-IFP might be helpfull in tracing the tissue of origin of intracerebral metastases of primary epithelial or myogenous tumors (Ramaekers et al., 1983; Miettinen et al., 1982). Conversely, extracerebral metastases of gliomas can be diagnosed by GFAP immunoreactivity.

3.3.7 References

1. **Anderton, B.H. (1981);**
   Intermediate filaments: A family of homologous structures.
   J. Muscle Res. Cell Motility 2:141-166.
2. **Bennett, G.S., Fellini, S.A., Holtzer, H. (1978):**
   Immunofluorescent visualization of 100 Å filaments in different cultured chick embryo cell types.
   Differentiation 12:71-82
3. **Bonnin, J.M., Rubinstein, L.J. (1984):**
   Immunohistochemistry of central nervous system tumors. Its contributions to neurosurgical diagnosis.
   J. Neurosurg. 60:1121-1133.
4. **Borit, A., Yung, A. (1983):**
   Glial fibrillary acidic protein (GFAP) and vimentin in brain tumors.
   J. Neuropathol. Exp. Neurol. 42: 308 (Abstract).
5. **Condeelis, J. (1981):**
   Microfilament-membrane interactions in cell shape and surface architecture. International Cell Biology 1980-1981.
   H.G. Schweiger ed. Springer Verlag (Berlin) pp 306-320.
6. **Dahl, D., Bignami, A., Weber, K., Osborn, M. (1981a):**
   Filament proteins in rat optic nerves undergoing Wallerian degeneration. II Localization of vimentin, the fibroblastic 100 Å filament protein in normal and reactive astrocytes.
   Exp. Neurol. 73:496-506.
7. **Dahl, D., Crosby, C., Bignami, A. (1981b):**
   Filament proteins in rat optic nerves undergoing Wallerian degeneration.
   Exp. Neurol. 71:421-430.
8. **Dahl, D., Rueger, D.C., Bignami, A., Weber, K., Osborn, M. (1981c):**
   Vimentin, the 57.000 molecular weight protein of fibroblast filaments, is the major cytoskeletal component in immature glia.
   Eur. J. Cell. Biol. 24:191-196.
9. **Dahl, D. (1983):**
   Immunohistochemical differences between neurofilaments in perikarya, dendrites and axons. Immunofluorescence study with antisera raised to neurofilament polypeptides (200 K, 150 K, 70 K) isolated by anion exchange chromatography.
   Exp. Cell. Res. 149: 397-408.
10. **DeArmond, S.J., Eng, L.F., Rubinstein, L.J. (1980):**
    The application of glial fibrillary acidic protein immunohistochemistry in neurooncology.
    Pathol. Res. Pract. 168:374-394.
11. **Deck, J.H., Eng, L.F., Bigbee, J., Woodcock, S.M. (1978):**
    The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors.
    Acta Neuropath. (Berlin) 42:183-190.
12. **Eng, L.F., Vanderhaegen, J.J., Bignami, A., Gerstl, B. (1971):**
    An acidic protein isolated from fibrous astrocytes.
    Brain Res. 28:351-354.

13. **Eng, L.F., Rubinstein, L.J. (1978):**
Contribution of immunohistochemistry to diagnostic problems of human cerebral tumors.
J. Histochem. Cytochem. 26:513-522.
14. **Eng, L.F., DeArmond, S.J. (1982):**
Immunochemical studies of astrocytes in normal development and disease.
Adv. Cell. Neurobiol. 3:145-171.
15. **Franke, W.W., Schmid, E., Osborn, M., Weber, K. (1978):**
Different intermediate-sized filaments distinguished by immunofluorescence microscopy.
Proc. Natl. Acad. Sci. USA 75:5034-5038.
16. **Franke, W.W., Schmid, E., Osborn, M., Weber, K. (1979a):**
Intermediate-sized filaments of human endothelial cells.
J. Cell. Biol. 81:570-580.
17. **Franke, W.W., Schmid, E., Winter, S., Osborn, M., Weber, K. (1979b):**
Widespread occurence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates.
Exp. Cell. Res. 123:25-46.
18. **Franke, W.W., Schmid, E., Schiller, D.L., Winter, S., Jarasch, E.D., Moll, R., Denk, H., Jackson, B.W., Illmensee, K. (1981):**
Differentiation-related patterns of expression of proteins of intermediate-sized filaments in tissues and cultured cells.
Cold Spring Harbor Symp. Quant. Biol. 46:431-453.
19. **Gabbiani, G., Kapanci, Y., Barazzone, P. Franke, W.W. (1981):**
Immunochemical identification of intermediate sized filaments in human neoplastic cells; A diagnostic aid for the surgical pathologist.
Am. J. Pathol 104:206-216.
20. **Goebel, H., Schlie, M., Moll, R. (1985):**
Application of immunohistological techniques to the diagnosis of gliomas.
In: Chemotherapy of gliomas, D. Voth and P. Krauseneck, eds. W. de Gruyter, Berlin, New York pp 35-42.
21. **Gould, V.E. (1985):**
The coexpression of distinct classes of intermediate filaments in human neoplasms.
Arch. Pathol. Lab. Med. 109: 984-985.
22. **Kepes, J.J., Rubinstein, L.J., Eng, L.F. (1979):**
Pleomorphic xanthoastrocytoma: a distinctive meningocerebral glioma of young subjects with relatively favorable prognosis. A study of 12 cases.
Cancer 44: 1839-1852.
23. **Lazarides, E. (1980):**
Intermediate filaments as mechanical integrators of cellular space.
Nature 283:249-256.
24. **Marsden, H.B., Kumar, S., Kahn, J., Anderton, B.J. (1983):**
A study of glial fibrillary acidic protein (GFAP) in childhood brain tumors.
Int. J. Cancer 31: 439-445.
25. **Miettinen, M., Lehto, V.-P., Bradley, R.A. (1982):**
Alveolar rhabdomyosarcoma. Demonstrations of the muscle type of intermediate filament protein, desmin, as a diagnostic aid.
Am. J. Pathol. 108: 246-251.

26. **Miettinen, M., Lehto, V.-P., Virtanen, I. (1984):**
Antibodies to intermediate filament proteins in the diagnosis and classification of human tumors.
Ultrastruct. Pathol. 7: 83-107.
27. **Moll, R., Franke, W.W., Schiller, D.L. (1982):**
The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells.
Cells 31: 11-24.
28. **Osborn, M., Geisler, N., Shaw, G., Sharp, G., Weber, K. (1981):**
Intermediate filaments.
Cold Spring Harbor Symp. Quant. Biol. 46:413-429.
29. **Osborn, M., Weber, K. (1983):**
Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology.
Lab. Invest. 48:372-394.
30. **Pruss, R.M., Mirsky, R., Raff, M.C., Thorpe, R., Dowding, A.J., Anderton, B.H. (1981):**
All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody.
Cell 27: 419-428.
31. **Ramaekers, F.C.S., Dunia, I., Dodemont, H.J., Benedetti, E.L., Bloemendal, H. (1982):**
Lenticular intermediate-sized filaments: Biosynthesis and interaction with plasma membrane.
Proc. Natl. Acad. Sci USA 79:3208-3212.
32. **Ramaekers, F.C.S., Puts J.J.G., Moesker O., Kant, A., Huysmans, A., Haag, D., Jap, P.H.K., Herman, C., Vooijs, G.P. (1983):**
Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumors; an overview.
Histochem. J. 15:691-713.
33. **Roessmann, U., Velasco, M.E., Gambetti, P., Autilio-Gambetti, L. (1983):**
Vimentin intermediate filaments are increased in human neoplastic astrocytes.
J. Neuropathol. Exp. Neurol. 42: 309 (Abstract).
34. **Roessmann, U., Velasco, M.E., Gambetti, P., Autilio-Gambetti, L. (1983):**
Neuronal and astrocytic differentiation in human neuroepithelial neoplasms.
J. Neuropathol. Exp. Neurol. 42: 113-121.
35. **Rubinstein, L.J. (1982):**
Supplement to tumors of the central nervous system. Atlas of Tumor Pathology, Series 2, Fascicle 6.
Washington D.C. Armed Forces Institute of Pathology, pp 21-31.
36. **Rubinstein, L.J. (1985):**
Embryonal central neuroepithelial tumors and their differentiating potential.
J. Neurosurg. 62: 795-805.

37. **Schlaepfer, W.W., Lee, V., Wu, H.L. (1981):**
Assessment of immunological properties of neurofilament triplet proteins.
Brain Res. 226: 259-272.
38. **Schnitzer, J., Franke, W.W., Schachner, M. (1981):**
Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system.
J. Cell. Biol. 90:435-447.
39. **Shaw, G., Weber, K. (1982):**
Differential expression of neurofilament triplet proteins in brain development.
Nature 298: 277-279.
40. **Trojanowski, J.Q. Lee, V., Pillsbury, N. (1982):**
Neuronal origin of human esthesioneuroblastoma demonstrated with anti-neurofilament monoclonal antibodies.
N. Engl. J. Med. 307: 159-161.
41. **Yung, W.K.A., Luna, M., Borit, A. (1985):**
Vimentin and glial fibrillary acidic protein in human brain tumors.
J. Neuro-Oncol. 3: 35-38.

PRODUCTION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) BY NEOPLASTIC CELLS: ADAPTATION TO THE MICROENVIRONMENT

Acta Neuropathol. (Berl) (1984) 64: 333-338.

Chapter 4

Production of Glial Fibrillary acidic protein (GFAP) by Neoplastic Cells: Adaptation to the microenvironment.

M.J.H.M. Herpers, H. Budka and D. McCormick.

Summary

In 80 specimens of human glioma the production of glial fibrillary acidic protein (GFAP) by tumor cells invading meninges or connective tissue was studied immunocytochemically by the PAP technique. In 38 of 55 cases of astrocytoma, glioblastoma, gliosarcoma, and oligoastrocytoma, GFAP immunoreactivity was greater in the invading cells as compared with the main part of the neoplasm. Fifty-eight percent of the astroglial tumors invading the leptomeninges, all astroglial tumors invading connective tissue and all gliosarcomas showed enhanced GFAP immunoreactivity of tumor cells getting in contact with collagenous tissue, whereas meningeal infiltrates of 25 non-astroglial tumors (oligodendroglioma, ependymoma, medulloblastoma) remained GFAP-negative like the main part of the respective tumors. In the majority of astroglial tumors an increase of GFAP immunoreactivity was found also in perivascular cells of the main part of the tumor.

It is concluded that glioma cells are capable of adapting their cytoskeleton to their micro-environment. Contact with dense collagenous tissue appears as an important factor able to induce an increased production of GFAP by adjacent glial cells.

Introduction

Glial fibrillary acidic protein (GFAP) has been isolated as a specific constituent protein of astroglial intermediate filaments. The protein is easily detectable by immunocytochemical methods and has therefore been used as a specific glial marker in studies of glial cytology in the normal and pathologically altered central nervous system (CNS) as well as in CNS neoplasms. Invasion of the leptomeninges by a glioma can induce a marked connective tissue reaction, mimicking in some instances a mesenchymal neoplasm. The immunohistochemical demonstration of GFAP in such tumors reveals their astroglial origin (Deck et al., 1978; Eng and Rubinstein, 1978; DeArmond et al., 1980; Eng and DeArmond, 1982).

When studying astrocytomas and glioblastomas with GFAP antisera, we observed an intensified immunostaining ("adaptation") of tumor cells invading or bordering mesenchymal tissue as compared to the noninvading part of the glial neoplasms. This phenomenon was briefly mentioned by one of us (Budka, 1983), but has not yet been studied systematically.

Table 1.
Increase of GFAP immunoreactivity of astroglial tumor cells at change of their microenvironment.

Diagnosis	Grading	N	N_1	N_2	Increase of immuno-reactivity of tumor cells invading		No change n	Perivascular increase of immuno-reactivity in main tumor tissue n
					lepto-meninges n	connective tissue n		
Astrocytoma	I, II	4	4		2	-	2	2
Anaplastic astrocytoma	III	22	19	3	12	3	7	11
Glioblastoma	IV	13	9	4	5	4	4	13
Giant cell glioblastoma	IV	1	1		1	-	-	-
Mixed glioblastoma-sarcoma	IV	6		6[a]	-	6	-	2
Mixed oligo-astrocytoma	II, III	9	7	2	3[b]	2[b]	4	2
Total		55	40	15	23	15	17	30

N, number of cases of each diagnosis; N_1, number of cases invading the leptomeninges; N_2, number of cases invading connective tissue (scar tissue or dura); n, number of cases showing the phenomenon.

[a] Number of gliosarcoma cases (glial tumor cells bordering densely fibrous sarcomatous portion were considered comparable to glioma cells invading connective tissue)
[b] Astroglial component.

Material and methods

Eighty neuroectodermal tumors with parts invading the meninges or central cicatrical fibrous tissue were investigated. All tumors were biopsies from the Institute of Neurology at the University of Vienna and were classified according to the WHO classification (Zülch, 1979).

Gliomas were categorised into four grades according to Kernohan et al. (1949) and Kernohan and Sayre (1952).

Fifty-five tumors were astrocytomas and glioblastomas or showed an astroglial component, including nine oligoastrocytomas and six mixed gliosarcomas. The non-astroglial tumor group consisted of 12 oligodendrogliomas, one ependymoma and 12 medulloblastomas. The tumor specimens were fixed in buffered 10% neutral formalin for 24-36 h and embedded and stored in paraffin. Storage from a few days to 6 years did not alter the immunoreactivity. Immunostained sections were compared with sections stained by haematoxylin-eosin (HE), Gomori, PTAH, and other stains. The immunostained sections were selected from representative tumor areas, and most of them had an area of several square centimetres (including necroses). However, the areas of tumor invading the meninges or fibrous cicatrisation varied greatly in size from sample to sample. The peroxidase-antiperoxidase (PAP) technique of Sternberger (1979) was used. The anti-GFAP serum was produced in rabbits after inoculation with partially purified GFAP form lamb brain (Delpech et al., 1978). Before use the antiserum was pre-absorbed with liver-plasma polymer prepared by the technique of Guesdon and Avrameas (1976). Commercial linking antibody (swine anti-rabbit IgG) and PAP complex (from rabbit) were purchased from DAKO, Copenhagen (Denmark) and normal swine serum from Gibco Europe, Glasgow (UK). Dilution experiments showed a dilution of the primary antiserum of 1:100 optimal for the incubation time of 30-45 min used. Specificity controls included substitution of the primary antiserum by non-immune rabbit serum or, in some instances, by anti-GFAP serum which had been preabsorbed with a preparation of purified lamb brain GFAP. The control sections did not show any specific immunoreactivity. As staining control, sections of a cerebral metastasis of a primary extracranial carcinoma surrounded by reactive gliosis were used. Counter-staining with haemalum was optional and used for a more precise localisation of the di-amino-benzidine (DAB) reaction product.

The slides were studied with special attention to (a) the intensity of immunostaining of tumor cells invading or bordering mesenchymal tissue, as compared with GFAP-positive tumor cells of the main part.

Differences in the intensity of immunoreactivity (i.e. darkness of the DAB reaction product) were evaluated by comparison of different parts in the same section, and (b) the number of immunoreactive tumor cells in areas invading or bordering mesenchymal tissue as compared with the relative number of GFAP positive cells elsewhere in the same specimen.

Results

Table 1 shows the incidence of increased GFAP immunoreactivity (increased staining intensity and/or an increased number of stained cells, Figs. 1-3) of astroglial tumor cells invading or bordering mesenchymal tissue. The grade of malignancy did not appear to be a major factor influencing that phenomenon. With regard to tumor types, changes of GFAP production were found among all diagnoses.

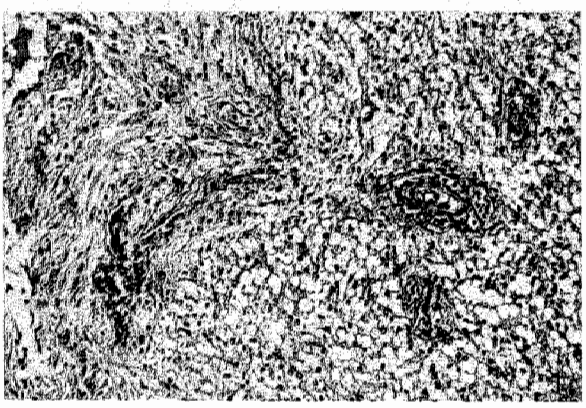


Fig. 1
Astrocytoma invading the leptomeninges, corresponding to the GFAP immunostained area shown in Fig. 2. HE; x135.

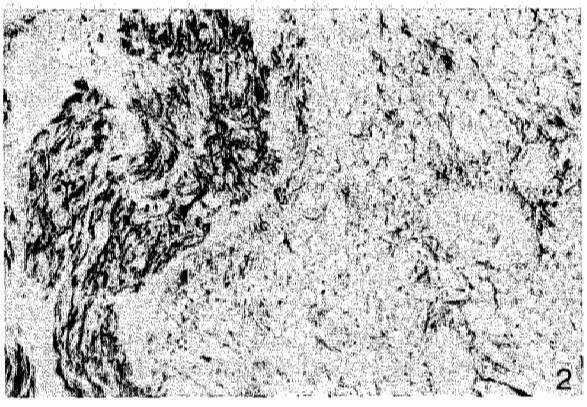


Fig. 2
GFAP immunoreactivity pattern of astrocytoma invading the leptomeninges. Increase in staining intensity of the invading tumor cells (left part) and a perivascular increase in number of immunoreacting cells within the non-invading tumor part (right part). GFAP-PAP counterstained with haemalum; x135.

All astroglial tumors invading fibrous connective tissue (six scar tissue and one dural invasion) showed an increase in the staining intensity of the invading tumor cell (Fig. 4). Tumor cells invading the leptomeninges or scar tissue showed a change in cell shape, many gemistocytic tumor elements becoming spindle-shaped.

The leptomeningeal tumor infiltrates of 11 oligodendrogliomas, one ependymoma and 12 medulloblastomas, and the tumor cells of one oligodendroglioma invading fibrous tissue were GFAP-negative like the main part of the respective tumors.
With regard to GFAP reactivity of the main part of the tumors, the GFAP reaction pattern of anaplastic astrocytomas was highly variable.
Many tumors showed very large GFAP-negative, usually cell-rich and small-celled areas with only focal weakly immunoreactive cells. However, large pleomorphic tumor cells were usually markedly positive. A diffuse fibrillary network of GFAP-positive cell processes was found in most areas. Focally or diffusely distributed GFAP-positive cell bodies were embedded in this GFAP-positive network. All glioblastomas showed GFAP-positive tumor cells. Usually, large tumor cells were intensely stained, while smaller tumor cells stained less intensely or were GFAP-negative. In contrast to the group of anaplastic astrocytomas, large areas without GFAP-positive tumor cells were not usually seen in this material. Both bodies and processes of peri-vascular tumor cells frequently showed an increase of immunoreactivity (Fig. 2).

Both anaplastic astrocytomas and glioblastomas showed GFAP-positive cells in mitosis, frequently of pathological shape. The giant cell glioma showed prominent GFAP-positive giant cells; however, completely GFAP-negative giant cells were also seen. The six gliosarcomas showed a mosaic pattern of islands containing GFAP-positive tumor cells, surrounded by GFAP-negative mesenchymal tumor tissue. In this tumor group there was an increase in the number of GFAP-positive tumor cells at the border of the glial tumor component and the mesenchymal tissue, as well as a perivascular increase of GFAP immunoreactivity (Fig. 5).

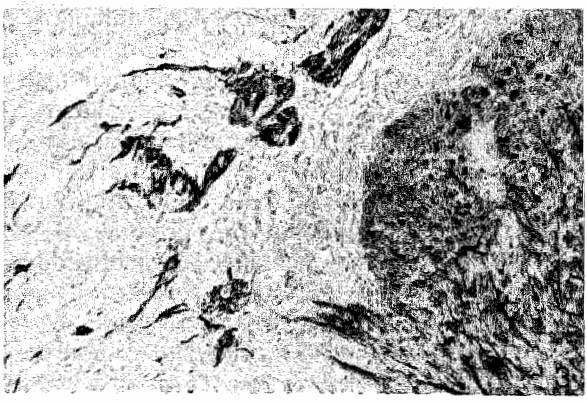


Fig. 3
GFAP immunostain of anaplastic astrocytoma invading the leptomeninges, showing an increase of immunoreactivity in the leptomeningeal part (left) as compared to the reaction pattern of the main tumor part (right). PAP counterstained with haemalum x135.

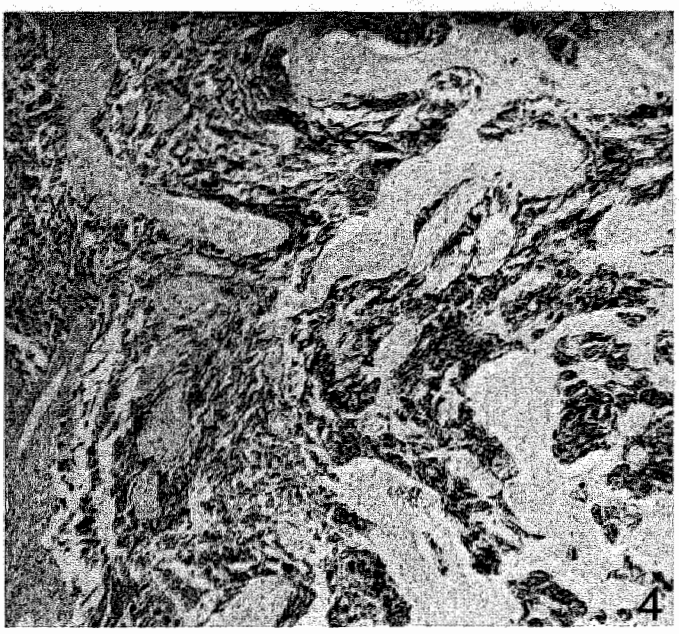


Fig. 4
Increase in GFAP immunostaining intensity of glioblastoma tumor cells invading collagenous scar tissue (left part). PAP counterstained with haemalum; x54.

Discussion

We present here evidence for a remarkable microenvironmental adaptation of GFAP production by neoplastic cells, which has not been described previously. All tumors invading compact fibrous tissues (scar tissue and dura mater) and all mixed gliosarcomas consistently showed an increase of GFAP immunoreactivity of infiltrating cells, whereas this phenomenon was seen somewhat less frequently, in about 58% of astroglial tumors invading the leptomeninges which have a less compact texture. Therefore, the presence of dense collagen appears to be an important factor in the induction of this phenomenon. In addition to instances of gliomatous invasion of mesenchymal tissues, an interface between glioma and mesenchymal tissue is always present in the tumor itself: the glial interface to vascular stroma. In a similar way to invading tumor cells, perivascular enhancement of GFAP immunoreactivity is frequently found here. Although this perivascular enhancement of GFAP immunoreactivity may represent in some places pre-existent non-neoplastic as well as reactive gliosis, the same pattern of immunoreacting tumor cells is also found around vessels deep within the tumor. This clearly demonstrates the consistency of interface enhancement of GFAP immunoreactivity, both in neoplastic and nontumorous tissue. A few cases of this series were immunostained also for S 100-protein without demonstrating enhancement patterns similar to GFAP; therefore, a general change of metabolism of invading or bordering tumor cells appears unlikely.

It might be argued that invading glial tumor cells could be compressed by adjacent connective tissue and result not only in a change of cell shape but in a

local increase of the GFAP concentration in a compressed "denser" cell cytoplasm. Such a conception, however, would expect also an increased concentration of other cytoplasmic constituents, such as S 100-protein; but this does not occur. Moreover, enhancement of GFAP immunoreactivity is seen also in cells bordering but not invading mesenchymal tissues; such cells are less likely to become "compressed".

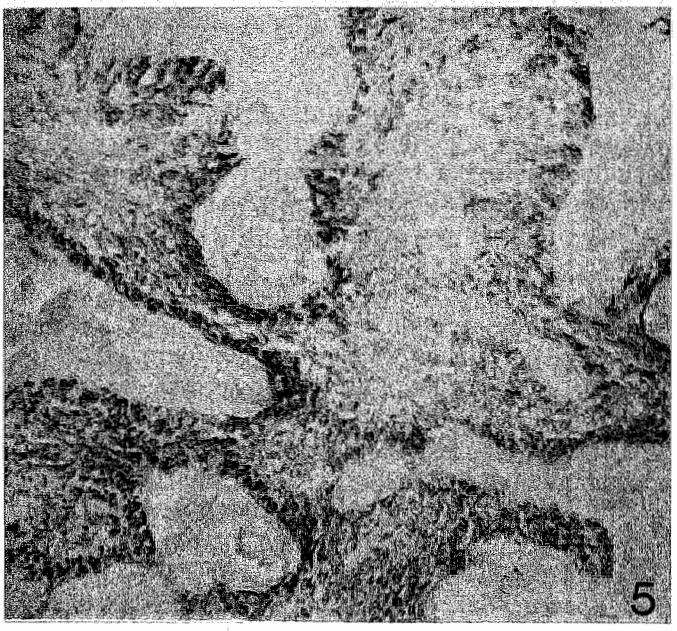


Fig. 5
Increase in number of GFAP immunoreactive tumor cells bordering connective tissue in a mixed gliosarcoma. PAP, no counterstain; x60.

In malignant gliomas, differences in GFAP immunoreactivity may be found between central and peripheral parts; the latter may be more differentiated and produce more GFAP, whereas the more anaplastic cell populations may be situated in the centre of the tumor. The enhanced GFAP production by tumor cells invading or bordering connective tissue is unlikely to result from such possible regional differences in GFAP production; invasion of meninges and connective tissue may occur both in peripheral and central parts of a glioma. Also the interface to vascular stroma is present in all parts of the tumor; invasion of scar tissue by tumor, showing regularly an increase of GFAP production by tumor cells, usually occurs in the cicatrised centre of necrotic gliomas.
In conclusion, our result suggest that glial cells, whether neoplastic, reactive or normal, have a tendency to strengthen their cytoskeleton when they contact mesenchymal tissue, including the formation of perivascular (end-feet) processes.
The absence of GFAP micro-environmental adaptation in oligodendroglioma, ependymoma and medulloblastoma, which invade the leptomeninges, clearly indicates the astroglial specificity of the phenomenon. However, more

ependymomas should be investigated because of the well known perivascular GFAP reactivity of this tumor (DeArmond et al., 1980; Deck et al., 1978; Duffy et al., 1978, 1979; Eng and DeArmond, 1982; Eng and Rubinstein, 1978; Tascos et al., 1982; Velasco et al., 1980), which was also confirmed in our laboratory. The increased GFAP production of tumor cells invading the leptomeninges in three mixed oligoastrocytomas, and invading fibrous connective tissue in two more cases of the same tumor type, appparently occured in the astroglial component; pure oligodendrogliomas invading the leptomeninges never showed that behaviour. The morphology of the invading tumor cells supported their astrocytic origin only in some examples.

Differences in the intensity of GFAP immunoreactivity of different tumor samples, even in the same tumor type and of the same grade, may be due also to multiple technical influences on the final dye intensity, e.g. section thickness, fixation procedures, development of the DAB reaction product and accessibility of antigenic sites. Only comparison of staining intensity of different cells within the same section seems reasonable. Reference to fixed staining intensity landmarks (Duffy et al., 1979), e.g. reactive astrocytes in the same section, is an important tool for proper interpretation of staining patterns of less intensely stained slides. The intensity of immunostaining has been found to correspond to micro-spectrophometric quantitations of the reaction product, confirming the visual observations (Duffy, 1982). Moreover, the intensity of immunostaining was seen to be proportional to the GFAP concentration by Delpech et al. (1978). Even without exact quantitating techniques, simple visual observation, as reported in this study, therefore appears useful to discriminate major changes of antigen concentration within the same immunostained slide.

It might be interesting to investigate if changes of cytoplasmic constituents as described here can be found also with other cytoskeletal proteins in gliomas and other tumors of the body. The whole scope of secondary adaptation of tumor cells to their microenvironment is far from being fully recognised up to now.

Acknowledgement.
Excellent technical assistance was given by Ms. Brigitte Windsperger.

References

1. **Budka, H. (1983):**
   Immunohistological demonstration of serum proteins and structural and viral antigens in paraffin sections of nervous tissues.
   Ann. NY Acad. Sci. 420: 176-184.
2. **DeArmond, S.J., Eng, L.F., Rubinstein, L.J. (1980):**
   The application of glial fibrillary acidic protein immuno-histochemistry in neurooncology.
   Pathol. Res. Pract. 168: 374-394.
3. **Deck, J.H., Eng, L.F., Bigbee, J., Woodcock, S.M. (1978):**
   The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors.
   Acta Neuropathol. (Berl.) 42: 183-190.
4. **Delpech, B., Delpech, A., Vidard, M.N., Girard, N., Tayot, J., Clement J.C., Creissard, P. (1978):**
   Glial fibrillary acidic protein in tumors of the nervous system.
   Br. J. Cancer 37: 33-40.
5. **Duffy, P.E. (1982):**
   Glial fibrillary acidic protein and induced differentiation of glia in vitro.
   J. Neurol. Sci. 53: 443-460.
6. **Duffy, P.E., Graf, L., Rapport, M.M. (1978):**
   Glial fibrillary acidic protein in gliomas using the immunoperoxidase method in human brain tumors.
   J. Neuropathol. Exp. Neurol. 37: 610.
7. **Duffy, P.E., Graf, L., Huang, Y.Y., Rapport, M.M. (1979):**
   Glial fibrillary acidic protein in ependymomas and other brain tumors.
   J. Neurol. Sci. 40: 133-146.
8. **Eng, L.F., DeArmond S.J. (1982):**
   Immunochemical studies of astrocytes in normal development and disease.
   Adv. Cell. Neurobiol. 3: 145-171.
9. **Eng, L.F., Rubinstein, L.J. (1978):**
   Contribution of immunohistochemistry to diagnostic problems of human cerebral tumors.
   J. Histochem. Cytochem. 26: 513-522.
10. **Guesdon, J.L., Avrameas, S. (1976):**
    Polyacrylamide-agarose beads for the preparation of effective immunoabsorbents.
    J. Immunol. Methods 11: 129-133.
11. **Kernohan, J.W., Sayre, G.P. (1952):**
    Tumors of the central nervous system. Armed Forces Institute of Pathology, Washington, DC.
12. **Kernohan, J.W., Mabon, R.F., Svien, H.J., Adson, A.W. (1949):**
    Simplified classification of gliomas.
    Proc. Mayo. Clin. 24: 71-75.
13. **Sternberger, L.A. (1979):**
    Immunocytochemistry, 2nd edn. Wiley, New York Chichester Brisbane Toronto.

14. **Tascos, N.A., Parr, J., Gonatas, N.K. (1982):**
Immunocytochemical study of the glial fibrillary acidic protein in human neoplasms of the central nervous system.
Hum. Pathol. 13: 454-458.
15. **Velasco, M.E., Dahl, D., Roessmann, U., Gambetti, P. (1980):**
Immunohistochemical localization of glial fibrillary acidic protein in human glial neoplasms.
Cancer 45: 484-494.
16. **Zülch, K.J. (1979):**
Histological typing of tumors of the central nervous system. International Histological Classification of Tumors. no 21. WHO, Geneva.

CO-EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN- (GFAP) AND VIMENTIN-TYPE INTERMEDIATE FILAMENTS IN HUMAN ASTROCYTOMAS

Acta Neuropathologica (accepted for publication).

Chapter 5

Co-expression of glial fibrillary acidic protein- (GFAP) and vimentin- type intermediate filaments in human astrocytomas

M.J.H.M. Herpers, F.C.S. Ramaekers, J. Aldeweireldt, O. Moesker, J. Slooff.

Summary

The expression of intermediate filament (IF) proteins was studied in seventy-one cases of malignant human astrocytoma and in seventeen cases of reactive gliosis, using immunocytochemical techniques with polyclonal and monoclonal antibodies to glial fibrillary acidic protein (GFAP) and vimentin.

In all cases of astrocytoma, varying in degree of malignancy from grade I to grade IV, co-expression of GFAP and vimentin was found. No change in vimentin- or GFAP-IF expression with increasing anaplasia was seen. In addition astrocytic cells in reactive gliosis showed simultaneous expression of GFAP and vimentin.

The intracellular distribution of these intermediate filament proteins differed. Vimentin was found to be located in a more juxta-nuclear position, whereas GFAP immunoreactivity showed a more intense staining of the cellular processes. Astrocytes in reactive gliosis behaved more or less like neoplastic cells. However thin cell processes of reactive astrocytes in the cortex and superficial white matter only contained GFAP immunoreactivity.

Simultaneous expression of GFAP and vimentin and their proportion in malignant and reactive glial cells are discussed in the light of earlier reports on the intermediate filament content of glial cells during development and maturation, in which vimentin precedes GFAP-expression. The existence of two separate (functional) IF systems in astroglia is suggested.

Keywords: astrocytoma - GFAP - immunocytochemistry - reactive gliosis - vimentin

Introduction

Intermediate-sized filaments are one of the three major fibrous systems which form the intracellular cytoskeleton in vertebrate cells. These highly insoluble structures are composed of five major classes of proteins, which have been shown to occur in a tissue specific manner (Franke et al., 1981; Osborn et al., 1981).

Tumors derived from different types of tissues in most cases retain their specific intermediate filament proteins (Osborn and Weber, 1983; Ramaekers et al., 1983[a]).

Co-expression of two different intermediate filament proteins has been described to occur in cultured cells (Franke et al., 1979), in some normal tissues and in a number of tumors (Ramaekers et al., 1985[a]). In most cases vimentin is

one of the two different types of intermediate filament proteins expressed. For astrocytes, glial fibrillary acidic protein (GFAP) is the specific intermediate-sized filament (IF)-system, formed in normal-, reactive-, tumor-, and cultured cells (Bignami et al., 1980; Eng and DeArmond, 1981).
In certain glial cells, such as immature glia and fibrous astrocytes, as well as in cultured glial cells both GFAP and vimentin are synthesized (Quinlan and Franke, 1983).
Stimulated by these reports, and by our recent finding that rhabdomyosarcomas may co-express vimentin and desmin, the number of desmin-positive cells decreasing with increasing dedifferentiation (Molenaar et al., 1985), we have investigated benign, including reactive, and malignant astrocytes, for their expression- and possible coexpression of vimentin and GFAP.

Materials and methods

Tissues:
Seventy-one astroglial tumors of different malignancy grades and 17 cases of reactive gliosis were investigated. The seventeen cases of reactive gliosis were autopsy specimens, and included brain abscesses, cases of Jacob-Creutzfeld disease, old encephalomalacies and a cerebral metastasis of an anaplastic carcinoma surrounded by reactive gliosis.
The tumors were classified according to the WHO classification (Zülch, 1979) and graded into four grades according to Kernohan et al. (1949) and Kernohan and Sayre (1952).
Tissue specimens had been routinely fixed in formalin and embedded in paraffin. In addition to the paraffin embedded material, tissue samples of 5 cases of astrocytoma were snap frozen in liquid nitrogen and used for immunocytochemical studies on frozen sections.
The following antisera were used in this study:

1. A rabbit antiserum directed against GFAP isolated from human spinal cord as described earlier (Ramaekers et al., 1983[a]); dilution 1:65 for immunofluorescence and up to 1:500 for the PAP-technique.
2. A rabbit antiserum raised against vimentin isolated from calf lens by preparative gel electrophoresis as described elsewhere (Ramaekers et al., 1981); dilution 1:20 for the fluorescence technique and 1:80 for the PAP-technique.
3. A monoclonal antibody to GFAP, obtained from Amersham, U.K. was used in a dilution of 1:650 (Gheuens et al., 1984).
4. A monoclonal antibody to bovine lens vimentin, obtained from EuroDiagnostics B.V. Apeldoorn, The Netherlands, was used in a dilution 1:50 (Ramaekers et al., 1985[b]).

Cross-reactivity of the antibodies with other intermediate filament proteins than the one they were raised against was excluded by immunoblotting assays (using cytoskeletal preparations from bovine lens, human spinal cord, human muscle and human skin).

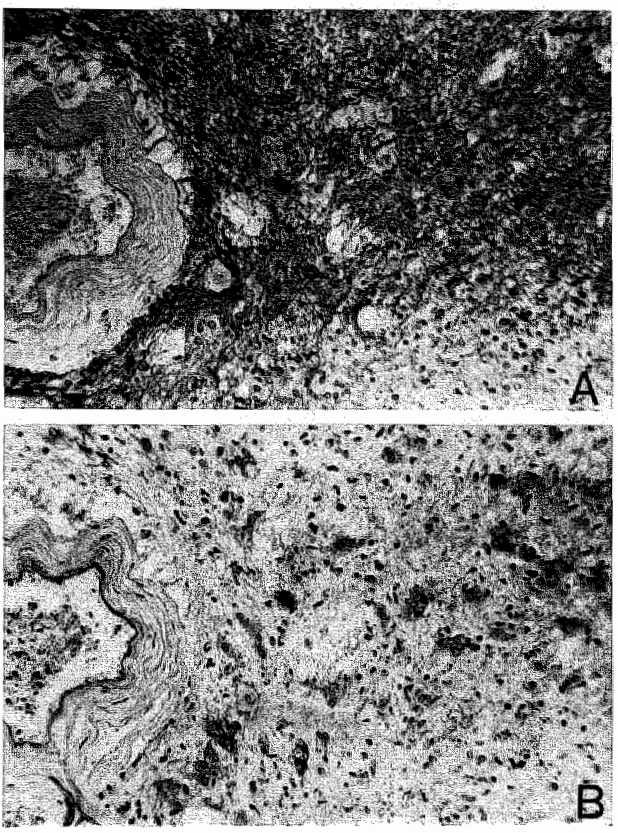


Fig. 1.
Different cellular distribution of GFAP and vimentin in astroglial tumor cells (Astrocytoma grade II).
a.) GFAP in perikaryon but also in even the smallest cell processes giving rise to a diffuse fibrillary network.
b.) Vimentin reactivity is more or less limited to the cell bodies. Paraffin sections x 150.

Indirect immunofluorescence and immunoperoxidase staining

Air-dried cryostat sections were fixed in 1% formalin in phosphate buffered saline (PBS) for 1 min. at roomtemperature and thereafter rinsed in PBS (2 x 10 min.).
All immune-incubation steps were performed at roomtemperature, unless specified otherwise. Furthermore, for the fluorescence techniques all antisera were diluted in 10% normal goat serum in PBS.
The first antibody was applied and the sections were incubated in a moist chamber for 45 min. After washing with PBS (two washes of 10 min. each), the fluoresceine-labeled second antibody (goat-anti-rabbit IgG or rabbit-anti-mouse IgG, both conjugated with fluorescein isothiocyanate (FITC); Nordic, Tilburg, The Netherlands) or sheepanti-mouse IgG labeled with Texas-Red (New England Nuclear, St. Albany, Boston) was added and the sections incubated for another 45 min. After a second series of washes in PBS, the sections were mounted with 50% glycerol in PBS or in Gelvatol (Monsanto, St. Louis,

Missouri, U.S.A.) and viewed with a Leitz Dialux 20 EB microscope equipped with epi-illumination using an appropriate filter combination. Pictures were taken on Tri-X film (Kodak) with an automatic camera using an ASA-setting of 400 or 800. Double-lable immunofluorescence was done essentially as described earlier (Ramaekers et al., 1983b). Briefly, the frozen sections were fixed in 1% formalin/PBS for 1 min. and rinsed in PBS (2 x 10 min.). Thereafter the sections were incubated with the monoclonal or the polyclonal vimentin antiserum for 45 min. and washed with PBS (2 x 10 min.).
Then the sections were incubated with the monoclonal or the polyclonal GFAP antiserum, depending on which type of vimentin antiserum had been applied. Both combinations (monoclonal vimentin antiserum with polyclonal GFAP antiserum or polyclonal vimentin antiserum with monoclonal GFAP antiserum) were thus used. After the third series of washes with PBS (2 x 10 min.) a mixture of FITC-conjugated-goat-anti-rabbit IgG (dilution 1:25) and Texas-Red-conjugated-sheep-anti-mouse IgG (dilution 1:50) was applied for 45 min. Then, after two final washing steps in PBS (10 min. each), the sections were mounted with 50% glycerol in PBS).
For the immunoperoxidase (PAP) technique, paraffin sections were deparaffinized using xylene (3 x 10 min.), and brought to PBS using a descending ethanol series, and subsequently treated with 1% hydrogen peroxide in methanol for 30 min. For the PAP technique all antisera were diluted in 10% normal swine serum (obtained from the CDL, Amsterdam) in PBS.

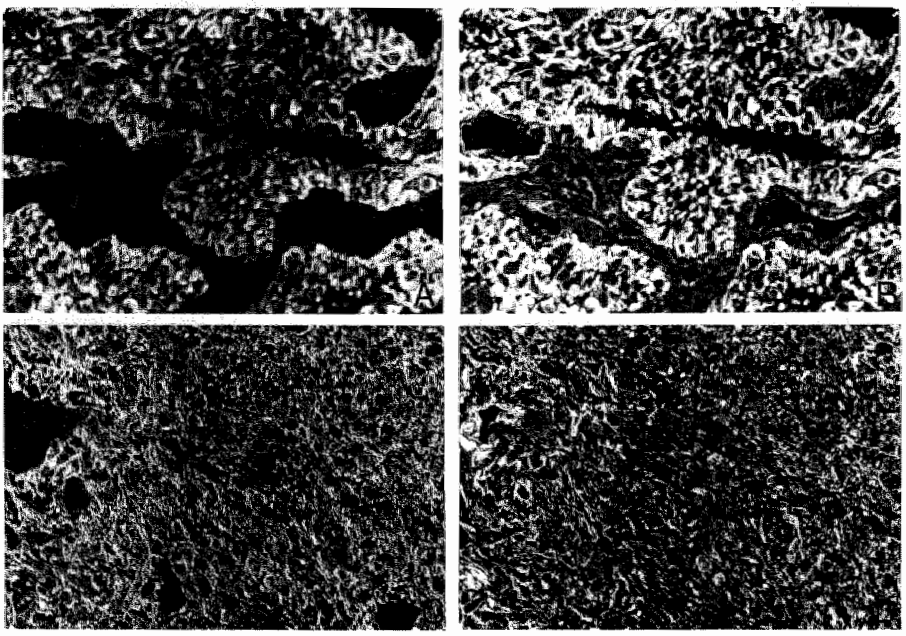


Fig. 2.
Double-label immunofluorescence micrographs of astrocytoma grades II (a, b) and IV (c, d) with the rabbit antiserum to GFAP (a) the monoclonal antibody to vimentin (b), the monoclonal antibody to GFAP (c) and the rabbit antiserum to vimentin (d). Note the intense staining of nearly all the tumor cells with the GFAP antisera (a, c) and the absence of staining of the stromal tissue (black). Whereas with vimentin antiserum both tissue components stain (b, d). Frozen sections x 150.

To reduce background binding, the sections were pre-incubated with 10% normal swine serum for 30 min. Subsequently the primary antiserum was applied and the sections were incubated overnight at 4°C., followed by another 2 h. at roomtemperature. After washing in PBS (3 x 10 min.), the sections were incubated 30 min. with swine-anti-rabbit IgG (Dako); dilution 1:30. After washing the sections were finally incubated (30 min.) with rabbit peroxidase-antiperoxidase complex (Dako); dilution 1:100. After washing, peroxidase activity was visualized using 3,3'-diaminobenzidine tetrahydrochloride (Sigma) for 5-10 min. Harris haematoxylin (1-3 min) was used as a counterstain. Control incubations using either PBS, preimmune sera, preabsorbed sera or non-reactive hybridoma culturing supernatants were performed on parallel sections.

Results

All 71 tumor specimens contained neoplastic cells showing immunoreactivity for GFAP as well as for vimentin.

However, the proportion of immunoreactive cells varied widely between the different cases, and even between different areas in one tissue section, most probably as a result of formalin fixation.

Neither the proportion of vimentin immunoreactive tumor cells, nor the intensity of the immunoreaction in the individual tumor cells showed any relation with the various grades of malignancy. Nearly all tumor specimens generally showed the same pattern of reactivity for GFAP and vimentin, again independent of the malignancy grade.

However, individual cases within all groups showed some differences. Most tumor cells reacted weakly with anti-vimentin-antiserum, compared to the more intense reactivity of the cells with the anti-GFAP-antiserum.

An exception to this observation were neoplastic giant-cells (which occured in 16 out of 27 grade III and IV astrocytomas), which often reacted more intensely with anti-vimentin antisera, in contrast to adjacent smaller protoplasmatic or fibrillary tumor cells.

When comparing the cellular distribution of the staining reaction of both antisera, most of the vimentin reactivity was found in a juxtanuclear position with very weak or absent staining only of proximal parts of cell processes. In contrast, GFAP was distributed more densely in the cellular processes over their full length, including very thin long processes.

In agreement with the latter observation was the persistent staining of a diffuse fibrillary network of tumor cell processes with anti-GFAP-antiserum which, on the other hand, did not show reactivity for vimentin (Fig. 1).

Perivascular enhancement (perivascular areas showing tumor cells with more intense immunoreactivity, or an increased number of immunoreactive tumor cells) occurred for GFAP only.

Figure 2 illustrates our findings of grade II and IV astrocytoma with the double label technique, using a combination of polyclonal anti-vimentin antiserum and monoclonal anti-GFAP antibody or polyclonal anti-GFAP antiserum with monoclonal anti-vimentin antibody, confirming our results with regard to co-expression of both intermediate filament systems in the same tumor cell. Co-expression of vimentin and GFAP was found in tumor cells in all grades of malignancy (Fig. 3).

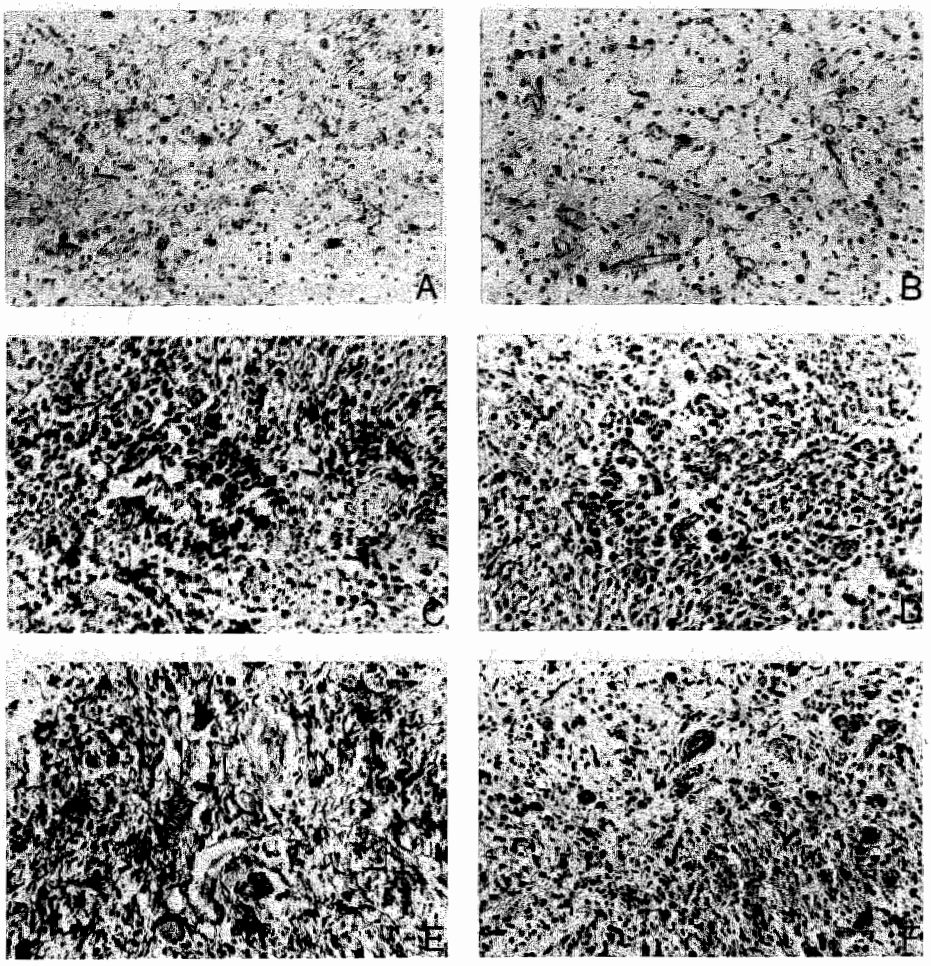


Fig. 3.
Immunoreactivity of GFAP antiserum (a, c, e) and vimentin antiserum (b, d, f) in astrocytomas grade II (a, b), grade III (c, d) and IV (e, f). Note the strong staining reactivity of cell processes with GFAP antiserum and restriction of vimentin reactivity to the cell body. Paraffin sections x 150.

In tumor specimens reactive astrocytes showed immunoreactivity for GFAP as well as vimentin in a pattern similar described for tumor cells, however, with even less intense vimentin immunoreactivity of the cell body (Fig. 4).
To confirm these observations, brain tissue samples with reactive gliosis were investigated. Also in these samples reactive astrocytes contained both GFAP- and vimentin intermediate filaments. In 15 out of 17 cases the large reactive astrocytes behaved more or less like neoplastic cells, showing a more juxtanuclear reactivity pattern for vimentin with staining only in the broad and proximal parts of the cell processes.
With the anti-GFAP antiserum especially the thin cell processes reacted intensely over their full length. Strikingly, the smaller reactive astrocytes in the cortex and superficial white matter were GFAP positive but vimentin negative.

Discussion

From earlier studies it is known that vimentin is a major constituent protein of immature glial cells (Dahl et al., 1981[a]; Schnitzer et al., 1981). This finding was recently underlined by the observation that relatively large amounts of vimentin positive cells occur in smears of neonatal rat brain and spinal cord (Björklund et al., 1984).
However, the ratio of vimentin to GFAP can vary during glial development and differentiation, the expression of vimentin preceding that of GFAP (Schnitzer et al., 1981; Shaw et al., 1981; Dahl et al., 1981[a]).
In glial precursor cells of rat brain white matter GFAP expression seems to be coordinated with the onset of myelination (Dahl et al., 1981[a]). Astrocytes in mature spinal cord and brain normally contain only GFAP but occasionally some vimentin can be detected (Dahl et al,, 1981[b], 1981[c]).
Furthermore, vimentin expression is also found in normal glial cells in tissue culture (Bennett et al., 1978[a] and 1978[b]), in rat glial tumor cell lines (Franke et al., 1978), in human glioma cell lines (Franke et al., 1978; Paetau et al., 1979), and in human gliomas in situ (Roessmann et al., 1983; Yung et al., 1985).
These latter studies and our present investigations show that vimentin expression in astrocytes (neoplastic and reactive) is not merely an artifact of tissue culture, as has been previously suggested, but also occurs in vivo.
Our results clearly show that upon malignant transformation of astrocytes, these cells initiate the synthesis of vimentin. This is concluded from the observation that astrocytes in normal cerebral cortex react only weakly if at all with vimentin antisera (Björklund et al., 1984), while virtually all investigated astrocytomas are vimentin positive.

The present study shows no clear change in vimentin content of neoplastic astrocytes during malignant progression (astrocytomas grade II to IV).
According to several authors GFAP expression by neoplastic astrocytes seems to be inversely proportional to the degree of anaplasia (Deck et al., 1978; Duffy et al., 1977; DeArmond et al., 1980; Eng and Rubinstein, 1978; Van der Meulen et al., 1978; Pasquier et al., 1983). Nevertheless we did not find such relation in the present study. As described in a previous report (Herpers et al., 1984) many tumors showed large GFAP-negative, usually cell-rich and small-celled areas with only few immunoreactive cells (usually with astrocytomas grade III). However, many large tumor cells, especially in glioblastomas, stained intensely

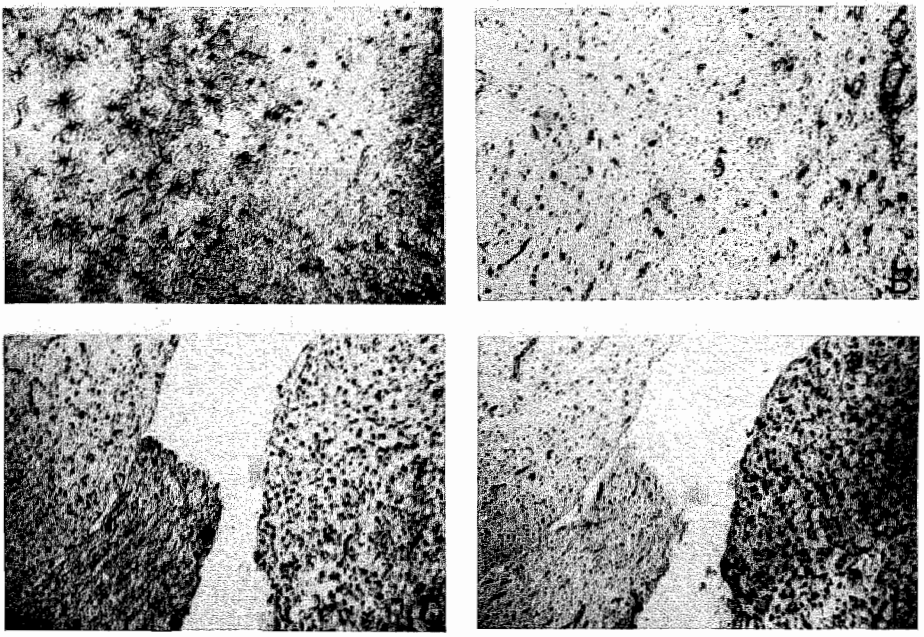


Fig. 4.
Reactive astrocytes in case of an astrocytoma grade IV (a, b) and astrocytoma grade III (c, d) (Tumorborder-regions).
a, c) Immunoreaction with anti-GFAP and
b, d) Immunoreaction with anti-vimentin.
Note the typical "spider-like-appearance" with staining of thin cell processes of the subcortical reactive astrocytes with GFAP-expression (a), which is absent with staining for vimentin (b). Furthermore the frequency and staining intensity of immunoreactive astrocytes is lower using vimentin-antiserum (compare a and b).
In the case shown in c and d reactive astrocytes (left-part) as well as tumor cells (right-part) are shown. Note the difference in immunoreactivity with the vimentin antibody between neoplastic and reactive astrocytes. Paraffin sections a, b x 150; c, d x 75.

with GFAP-antisera. The latter finding led us to the conclusion that there is little or no difference in GFAP expression of neoplastic astrocytes of various malignancy grades. The increased vimentin immunoreactivity of the giant cells, which normally only occur in higher grade astrocytomas, may be interpreted as a special cytoskeletal feature of these tumor cells.
Interesting, however, is the observation of immunoreactivity of reactive astrocytes with vimentin, antisera. Though neoplastic cells showed a far more intense immunoreactivity with vimentin, reactive astrocytes were not completely negative.
On the contrary, GFAP expression of reactive astrocytes seems to be increased. For an experienced neuropathologist, this phenomenon may be of some help for the differentiation between neoplastic and reactive astrocytes, especially in those tumor areas which border or infiltrate surrounding brain tissue with reactive gliosis. Since vimentin can be observed in vivo in tumor cells as well as in reactive astrocytes and in cultured glial cells it is not clear at this moment what

initiates vimentin synthesis in these cells. Vimentin was mainly found in a juxtanuclear position in neoplastic as well as in reactive cells, with occasionally faint staining in proximal cell processes. GFAP, on the other hand, was found in the cell bodies as well as in all extensions, even when they were very thin, suggesting the existence of two separate systems of intermediate filaments in astroglia (Dahl et al., 1981[a]). This hypothesis is, however, in contrast with the findings in cultured glioma cells, in which GFAP and vimentin have been described to (partly) colocalize and to form heteropolymer filaments (Quinlan and Franke, 1983; Sharp et al., 1982). In this respect one should keep in mind that formalin fixation may destroy intermediate filament protein epitopes. This may be especially true for cytokeratins and vimentin (Ramaekers et al., 1983[a]). As a result the antibody to vimentin applied to paraffin sections may exhibit a lower sensitivity than the GFAP-antiserum.
Thus cells or cell compartments which contain low amounts of vimentin may appear negative in paraffin sections, as seen for example in the cell extensions. However, this phenomenon is extremely reproducible and also observed in tumors in which vimentin staining is strong in both tumor cells and in the stromal components, suggesting to us that we are not dealing with a fixation artifact. These differences in intracellular distribution have also been mentioned in earlier studies (Dahl et al., 1981c; Schnitzer et al., 1981), and may be related to differences in the function of the two IF-systems.
From earlier reports (Eng and DeArmond, 1982) it is known that in vitro GFAP filaments, which participate in the formation of a cytoskeleton, first appear in the perinuclear region and as maturation progresses extend radially to the cell periphery. This is followed by cytoplasmic cavitation which ultimately gives rise to "the formation of mature multipolar astrocytes with filament packed cellular processes".

Acknowledgements

The authors wish to thank Prof. Dr. F. Bosman (University of Limburg, NL) and Dr. H. Budka (University of Vienna) for stimulating discussions and critical reviewing of the manuscript. Yvonne Stammes is acknowledged for excellent secretarial help.

References

1. **Bennett, G.S., Fellini, S.A., Croop, J.M., Otto, J., Bryan, J., Holtzer, H. (1978[a]):**
   Differences among the 100 Å filament subunits from different cell types.
   Proc. Natl. Acad. Sci. U.S.A. 75: 4364-4368.
2. **Bennett, G.S., Fellini, S.A., Holtzer, H. (1978b):**
   Immunofluorescent visualization of 100 Å filaments in different cultured chick embryo cell types.
   Differentiation 12: 71-82.
3. **Bignami, A., Dahl., D., Rueger, C. (1980):**
   Glial fibrillary acidic (GFA) protein in pathological conditions. Vol. 1, p 285-319, S. Fedoroff and L. Hertz, eds., Academic Press, New York, London.
4. **Björklund, H., Eriksdotter-Nilsson, M., Dahl, D., Olson, L. (1984):**
   Astrocytes in smears of CNS tissues as visualized by GFA- and vimentin-immunofluorescence.
   Med. Biol. 62: 38-48.
5. **Dahl, D. Rueger, D.C., Bignami, A., Weber, K., Osborn, M. (1981a):**
   Vimentin, the 57,000 dalton protein of fibroblast filaments is the major cytoskeletal component in immature glia.
   Eur. J. Cell Biol. 124: 191-196.
6. **Dahl, D., Crosby, C.J., Bignami, A. (1981b):**
   Filament proteins in rat optic nerves undergoing Wallerian degeneration.
   Exp. Neurol. 71: 421-430.
7. **Dahl, D., Bignami, A., Weber, K., Osborn, M. (1981[c]):**
   Filament proteins in rat optic nerves undergoing Wallerian degeneration. Localization of vimentin, the fibroblastic 100 Å filament protein, in normal and reactive astrocytes.
   Exp. Neurol. 73: 496-506.
8. **DeArmond, S.J., Eng, L.F., Rubinstein, L.J. (1980):**
   The application of glial fibrillary acidic protein immuno-histochemistry in neurooncology.
   Pathol. Res. Pract. 168: 374-394.
9. **Deck, J.H., Eng, L.F., Bigbee, J., Woodcock, S.M. (1978):**
   The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors.
   Acta Neuropathol. (Berl.), 42: 183-190.
10. **Duffy, P.E., Graf, L. (1977):**
    Identification of glial fibrillary acidic protein by the immunoperoxidase method in human brain tumors. J. Neuropathol.
    Exp. Neurol. 4: 645-652.
11. **Eng, L.F., DeArmond, S.J. (1981):**
    Glial fibrillary acidic (GFA) protein immunocyto-chemistry in development and neuropathology.
    In: Glial and neuronal cell biology, p 65-79. E.A. Vidro, Ed.: Alan R. Liss Inc., New York.

12. **Eng, L.F., DeArmond, S.J. (1982):**
Immunochemical studies of astrocytes in normal development and disease.
Adv. Cell Neurobiol. 3: 145-171.
13. **Eng, L.F., Rubinstein L.J. (1978):**
Contribution of immunochemistry to diagnostic problems of human cerebral tumors.
J. Histochem. Cytochem. 26: 513-522.
14. **Franke, W.W., Schmid, E., Osborn, M., Weber, K. (1978):**
Different intermediate-sized filaments distinguished by immunofluorescence microscopy.
Proc. Natl. Acad. Sci. U.S.A. 75: 5034-5038.
15. **Franke, W.W., Schmid, E., Osborn, M., Weber, K. (1979):**
Widespread occurence of intermediate-sized filaments of the vimentin type in cultured cells from diverse vertebrates.
Exp. Cell. Res. 123: 25-46.
16. **Franke, W.W., Schmid, E., Schiller, D.L., Winter, S., Jarasch, E.D., Moll, R., Denk, H., Jackson, B.W., Illmensee, K. (1981):**
Differentiation-related patterns of expression of proteins of intermediate-sized filaments in tissues and cultured cells.
Cold Spring Harbor Symp. Quant. Biol. 46:431-453.
17. **Gheuens, J., de Schutter, B., Noppe, M., Löwenthal, A. (1984):**
Identification of several forms of glial fibrillary acidic protein, or alpha-albumin by a specific monoclonal antibody.
J. Neurochem. 43: 964-970.
18. **Herpers, M.J.H.M., Budka, H., McCormick, D. (1984):**
Production of glial fibrillary acidic protein (GFAP) by neoplastic cells: adaptation to the micro-environment.
Acta Neuropathol. (Berl.) 64: 333-338.
19. **Kernohan, J.W., Mabon, R.F., Svien, H.J., Adson, A.W. (1949):**
Simplified classification of gliomas.
Proc. Mayo Clin. 24: 71-75.
20. **Kernohan, J.W., Sayre, G.P. (1952):**
Tumors of the central nervous system.
Armed Forces Institute of Pathology, Washington D.C.
21. **van der Meulen, J.D.M., Houthoff, H.J., Ebels, E.J. (1978):**
Glial fibrillary acidic protein in human gliomas.
Neuropathol. Appl. Neurobiol. 4: 177-190.
22. **Miller, R.H., Raff, M.C. (1984):**
Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct.
J. Neurosci. 4, 2: 585-592.
23. **Molenaar, W.M., Oosterhuis, J.W., Oosterhuis, A.M., Ramaekers, F.C.S. (1985):**
Mesenchymal and muscle specific intermediate filaments, vimentin and desmin, as markers for differentiation in childhood rhabdomyosarcomas.
Human Pathol. 16: 838-843.
24. **Osborn, M., Geisler, N., Shaw, G., Sharp, G., Weber, K. (1981):**
Intermediate filaments.
Cold Spring Harbor Symp. Quant. Biol. 46: 413-419.

25. **Osborn, M., Weber, K. (1983):**
Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology.
Lab. Invest. 48: 372-394.
26. **Pasquier, B., Lachard, A., Pasquier, D., Couderc, P., Delpech, B., Courel, M.N. (1983):**
Protéine gliofibrillaire acide (GFA) et tumeurs nerveuses centrales. Etude immunohistochimique d'une série de 107 cas. IIe Partie.
Ann. Pathol. 3: 203-211.
27. **Paetau, A., Virtanen, I., Stenman, S., Kurke, P., Linder, E., Valeri, A., Westermark, B., Dahl, D., Holtia, M. (1979):**
Glial fibrillary acidic protein and intermediate filaments in human glioma cells.
Acta Neuropathol. (Berl.) 47: 71-74.
28. **Quinlan, R.A., Franke, W.W. (1983):**
Molecular interactions in intermediate filaments revealed by chemical cross-linking. Heteropolymers of vimentin and glial filament protein in cultured human glioma cells.
Eur. J. Biochem. 132: 477-484.
29. **Raff, M.C., Miller, R.H., Noble, M. (1983[a]):**
A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium.
Nature 303: 390-396.
30. **Raff, M.C., Abney, E.R., Cohen, J., Lindsay, R., Noble, M. (1983[b]):**
Two types of astrocytes in cultures of developing rat white matter: Differences in morphology, surface gangliosides, and growth characteristics.
J. Neurosci. 3: 1289-1300.
31. **Ramaekers, F.C.S., Puts, J.J.G., Moesker, O.J., Jap, P.H.K., Vooijs, G.P. (1981):**
Use of antibodies to intermediate filaments in the characterization of human tumors.
Cold Spring Harbor Symp. Quant. Biol. 46: 331-339.
32. **Ramaekers, F.C.S., Puts, J.J.G., Moesker, O., Kant, A. Huysmans, A., Haag, D., Jap, P.H.K., Herman, C., Vooijs, G.P. (1983[a]):**
Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumors; an overview.
Histochemical Journal 15: 691-713.
33. **Ramaekers, F.C.S., Haag, D., Kant, A., Moesker, O., Jap, P.H.K., Vooijs, G.P. (1983[b]):**
Coexpression of keratin and vimentin-type intermediate filaments in human metastatic carcinoma cells.
Proc. Natl. Acad. Sci. U.S.A. 80: 2618-2622.
34. **Ramaekers, F.C.S., Broers, J. Vooijs, P. (1985[a]):**
Immunocytochemistry of intermediate filament proteins.
In: Immunocytochemistry, practical application in pathology and biology - second edition (J.M. Polak and S. van Noorden, eds.). J. Wright and sons Ltd., London (in press).

35. **Ramaekers, F.C.S., Feitz, W., Moesker, O., Schaart, G., Herman, C., Debruyne, F., Vooijs, P. (1985[b]):** Antibodies to cytokeratin and vimentin in testis tumor diagnosis.
Virchows Arch. A (Pathol. Anat. and Histopathol.) 408: 127-142.
36. **Roessmann, N., Velasco, M.E., Gambetti, P., Autilio-Gambetti, L. (1983) (abstract):**
Vimentin intermediate filaments are increased in human neoplastic astrocytes.
J. Neuropathol. Exp. Neurol. 42: 309.
37. **Schnitzer, J., Franke, W.W., Schachner, M. (1981):**
Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous sytem.
J. Cell Biol. 90: 435-447.
38. **Shaw, G., Osborn, M., Weber, K. (1981):**
An immunofluorescence microscopical study of the neurofilament triplet proteins, vimentin and glial fibrillary acidic protein within adult rat brain.
Eur. J. Cell Biol. 26: 68-82.
39. **Sharp, G., Osborn, M., Weber, K. (1982):**
Occurence of two different intermediate filament proteins in the same filament in situ within a human glioma cell line.
Exp. Cell Res. 144: 385-395.
40. **Sternberger, L.A. (1979):**
Immunocytochemistry, 2nd edn. Wiley, New York, Chichester, Brisbane, Toronto.
41. **Velasco, M.E., Dahl, D., Roessmann, N., Gambetti, R. (1980):**
Immunohistochemical localization of glial fibrillary acidic protein in human glial neoplasms.
Cancer 45: 484-494.
42. **Wang, E., Cairncross, J.G., Liem, R.K. (1984):**
Identification of glial filament protein and vimentin in the same intermediate filament system in human glioma cells.
Proc. Natl. Acad. Sci. U.S.A. 81: 2102-2106.
43. **Yung, W-K.A., Luma, M., Borit, A. (1985):**
Vimentin and glial fibrillary acidic protein in human brain tumors.
J. Neuro-Oncol. 3: 35-38.
44. **Zülch, K.J. (1979):**
Histological typing of tumors of the central nervous system.
International Histological Classification of Tumors, no. 21, W.H.O., Geneva.

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IN OLIGODENDROGLIAL TUMORS: GLIOFIBRILLARY OLIGODENDROGLIOMA AND TRANSITIONAL OLIGOASTROCYTOMA AS SUBTYPES OF OLIGODENDROGLIOMA.

Acta Neuropathol. (Berl.) (1984) 64: 265-272.

Chapter 6

Glial fibrillary acidic protein (GFAP) in Oligodendroglial tumors: gliofibrillary oligodendroglioma and transitional oligoastrocytoma as subtypes of oligodendroglioma.

M.J.H.M. Herpers, and H. Budka.

Summary
Immunoreactivity to glial fibrillary acidic protein (GFAP) is mainly regarded as a sign of astroglial histogenesis and/or differentiation.
The presence of astrocytes in oligodendrogliomas is a well known phenomenon; in addition, GFAP-positive neoplastic oligodendrocytes have also been described but have not yet been studied systematically.
Using an anti-GFAP serum in the peroxidase-antiperoxidase (PAP) technique, 50 oligodendrogliomas and 16 mixed oligodendrocytomas were investigated; they had been diagnosed by routine histological stains. In half of all oligodendrogliomas, and only in a few (12%) of the mixed oligoastrocytomas, GFAP-positive oligodendrocytes were found in some areas of the classical honey-comb texture with a prominent vascular stroma. The term 'gliofibrillary oligodendrocyte' (gfoc) is proposed for these immunoreactive cells. The existence of a tumor cell combining morphological characteristics of oligodendroglia with GFAP production in its cytoplasm may be considered analogous to transient GFAP expression by myelin-forming glia during normal development (Choi and Kim, 1984), thus suggesting the return to a foetal behaviour by some neoplastic oligodendrocytes. Three tumors of the present series consisted largely of gfocs and, therefore, may be termed gliofibrillary oligodendrogliomas. In about 32% of all oligodendrogliomas, but only once in the mixed tumor group, a gradual morphological transition from gfocs to gemistocytic astrocytes was observed. This suggested a transitional cell type or transitional oligoastrocytoma as a further subtype of oligodendroglioma, including one example in which minigemistocytes dominated over gfocs ("minigemistocytoma"). These oligodendroglioma subtypes with GFAP-containing cells are different from the mixed oligoastrocytoma which is a tumor of two distinct and non-transitional cell populations.

Key-words
GFAP - Oligodendroglioma - Mixed oligoastrocytoma - Tumor differentiation - Immunocytochemistry.

Introduction
Glial fibrillary acidic protein (GFAP) has been isolated as a specific constituent protein of astroglial intermediate filaments. The protein is easily detectable

immunohistochemically and, therefore, has been used as a specific glial marker for studies of glial cytology in normal and pathological central nervous system (CNS) and in CNS neoplasms. The presence of astrocytes in oligodendrogliomas is well known; they may be regarded as reactive elements or as neoplastic components of mixed oligoastrocytomas. Using GFAP antisera, different results have been described by various investigators with regard to the existence of GFAP immunoreactive neoplastic cells. GFAP negative oligodendrogliomas were reported by Deck et al. (1978), Eng and Rubinstein (1978), Tascos et al. (1982), and Velasco et al. (1980), whereas Van der Meulen et al. (1978), DeArmond et al. (1980), Ishida et al. (1982), and Kepes and Meneses (1982) described tumors containing GFAP positive oligodendroglial neoplastic cells. Most of these immunohistochemical investigations were part of extensive surveys of GFAP immunoreactivity in all types of glioma including limited numbers of oligodendroglial tumors. The present study of a large series of oligodendrogliomas and mixed oligoastrocytomas was therefore designed to study systematically the frequency of GFAP positive neoplastic oligodendrocytes and their relation to mixed oligoastrocytomas.

Materials and methods

Fifty oligodendrogliomas were studied, including two recurrent tumors and two double recurrences in 44 patients; 16 mixed oligodendroglial-astroglial neoplasms included one recurrent tumor, and, in HE- and PTAH stained sections, usually showed separate, but occasionally intermingling areas of pure oligodendroglioma and astrocytoma. All the tumors were biopsies from the collection of the Institute of Neurology at the University of Vienna and classified according to the WHO classification (Zülch, 1979) on HE- and PTAH stained sections. Grading of the gliomas was in accordance with the grades proposed by Kernohan et al. (1949) and Kernohan and Sayre (1952). Ependymatous features were not present. The tumor specimens were fixed in 10% neutral buffered formalin for 24-36 h usually and embedded and stored in paraffin. Prolonged storage did not alter the immunoreactivity; the storage times ranged from a few days to 9 years.

The immunostained slides were selected from areas considered representative of the tumor and, in most instances, covered an area of several square centimetres.

The peroxidase antiperoxidase (PAP) technique according to Sternberger (1979) was used.

The anti-GFAP serum was produced in the rabbit and kindly provided by Dr. D. McCormick, Belfast (Northern Ireland); more details of this serum are given elsewhere (Herpers et al., 1984). Commercially available linking antibody (swine anti rabbit IgG) and PAP complex (from rabbit) were purchased from DAKO, Copenhagen (Denmark) and normal swine serum from Gibco Europe, Glasgow (UK). In dilution experiments, a 1% dilution of the primary antiserum was found optimal for the incubation time of 30-45 min. Specificity controls included substitution of the primary antiserum by non-immune rabbit serum or, in some cases, by the anti-GFAP serum which had been pre-absorbed with purified GFAP. All these control sections showed no specific immunoreactivity. Sections in which reactive gliosis surrounded a cerebral metastasis from a primary extracranial carcinoma were used as a control for staining.

Counterstaining with haemalum was optional and used for a more precise localisation of the di-amino-benzidine (DAB) reaction product.

Results

GFAP positive cells were considered to be oligodendroglial when they presented a clear halo around a round chromatin-rich nucleus in an area with the classical characteristics of a honey-comb texture of uniform sheets intersected by a regular vascular stroma. This GFAP-immunoreactive oligodendroglioma cell was called 'gliofibrillary oligodendrocyte' (gfoc) in the present series because of its cytology and immunoreactivity (Figs. 1A,B). Generally, only a small perinuclear rim showed GFAP positivity. Transitional cell forms were characterized by a gradual increase in breadth of the GFAP-positive cytoplasm (Figs. 1C,D; 2A). With eccentric nuclei, such cells frequently resembled a miniature form of gemistocytes ('minigemistocytes', Kepes and Meneses, 1982; Fig. 2C). There was also further transition to large positive tumor cells of classical gemistocytic appearance (Fig. 2B). Transitional cells were encountered in many tumors in the oligodendroglioma group, but found only once in the mixed oligoastrocytoma group.

Table I.
The incidence of gfocs and transitional cells in different malignancy groups of oligodendroglioma in 50 cases.

Grading	No. of cases	gfocs	transitional cells	gfocs absent
I + II	41	20/41	13/41	21/41
III	9	5/ 9	3/ 9	4/ 9
Total	50	25/50	16/50	25/50

gfocs, gliofibrillary oligodendrocytes = GFAP-positive oligodendroglial tumor cells.

Table 2.
The incidence of gfocs and transitional tumor cells in the oligodendroglial component of mixed oligoastrocytomas in 16 cases.

Grading	No. of cases	gfocs	transitional cells	gfocs absent
I + II	8	2/ 8	1/ 8	6/ 8
III	8	-	-	8/ 8
Total	16	2/ 8	1/ 8	14/16

gfoc, gliofibrillary oligodendrocyte = GFAP-positive oligodendroglial tumor cell.

Tables 1 and 2 show the incidence of gfocs and transitional GFAP reactive cell forms among the different malignancy grades of the two tumor groups. The incidence of GFAP positive cells did not relate to the grade of malignancy.

The density of gfocs in the tumor specimens ranged from a few scattered cells in the majority of cases to tumors with large areas composed entirely of gfocs and to four tumors almost completely composed of GFAP positive cells (gfocs and transitional cells). In the latter four cases, the HE slides showed the classical honey-comb structure of the oligodendroglioma intersected by a prominent vascular stroma (Figs. 1B,D). Three of these tumors were not stained by PTAH. One "minigemistocytoma" of these tumors showed large areas with immunoreacting cells of minigemistocytic shape dominating over gfocs (Fig. 2C); some minigemistocytic cells were negative for GFAP. The PTAH stain of this case showed only a few scattered fibrils in prominently GFAP positive areas. Distribution of gfocs was usually randomised; two tumors showed gfocs more frequently in perivascular areas. Large parts of pure oligodendrogliomas as well as the oligodendroglial part of mixed tumors were frequently without any GFAP immunoreactivity. However, a diffuse fibrillary GFAP positive network of cell processes was also frequently found around GFAP negative oligodendroglial tumor cells, thus outlining the classical honey-comb pattern (Fig. 2D).
The astroglial tumor component of the mixed astroglial-oligodendroglial neoplasm ranged from relatively few intensely stained astrocytic cells to some tumors very rich in labelled astrocytes.

Discussion

In this study by light microscopy, cells with typical features of oligodendrocytes were found to contain GFAP in about half of the pure oligodendrogliomas and less frequently in oligodendroglial parts of some oligoastrocytomas. Van der Meulen et al. (1978) found GFAP-positive neoplastic oligodendrocytes in only three of 13 anaplastic oligodendrogliomas (grade III). Since GFAP-positive tumor cells were not found in benign oligodendrogliomas of their series they postulated a certain degree of malignancy (anaplasia) as a necessary factor for the production of GFAP by neoplastic oligodendrocytes, and a transition to astrocytic (gemistocytic) elements. In the present study, GFAP positive neoplastic oligodendrocytes were found independent of the grade of malignancy.
In contrast to astrocytic tumors, which regularly show an increase of GFAP immunoreactivity in perivascular areas (Herpers et al., 1984), only two oligodendroglial tumor exhibited this phenomenon, whereas all other GFAP positive oligodendrogliomas showed a randomised distribution of gfocs.

How are these GFAP positive cells of oligodendrogliomas to be interpreted? Table 3 summarises various opinions found in the literature. There have been three main interpretations: First, GFAP-positive cells in oligodendrogliomas may be a type of gemistocytic astrocytes in which glial fibrils may not be demonstrated by classical stains for glia (DeArmond et al., 1980; Rubinstein, 1972). Second, they may represent an intermediate or transitional tumor cell from oligodendroglial to astroglial tumor (Van der Meulen et al., 1978). The third interpretation is the possibility of a bipotential glial precursor cell (Raff et al., 1983). A further explanation may be the constant or temporary production of GFAP by neoplastic oligodendrocytes as sign of reverting to the fetal behaviour of oligodendroglia (Choi and Kim, 1984) without necessarily implying an astrocytic histogenesis.

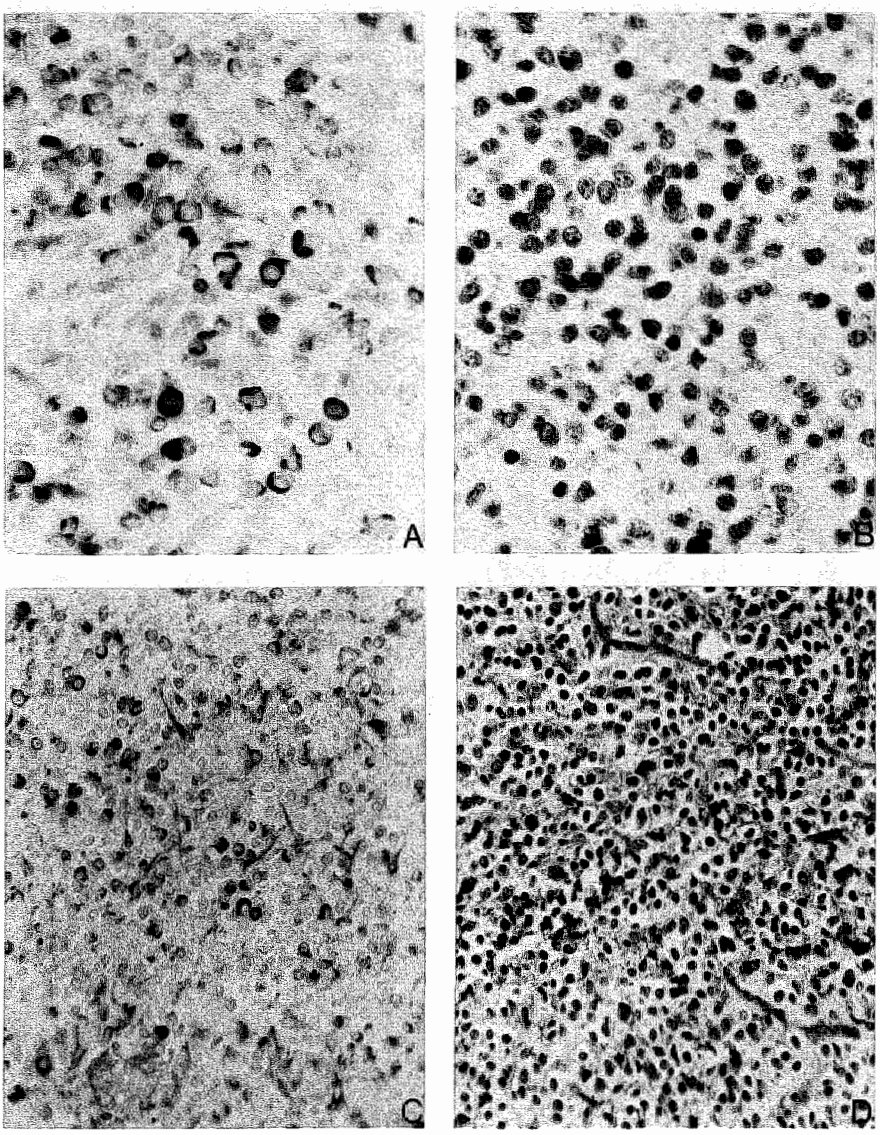


Fig. 1
A, B. Gliofibrillary oligodendroglioma.Numerous tumor cells with a narrow GFAP-positive cytoplasmic rim (A) in an area with classical features of oligodendroglioma in HE stain (B); x 540. C, D. Transitional cell type. Numerous tumor cells with a variable breadth of GFAP-positive cytoplasm (C) in an area of the classical honey-comb texture in HE stain (D); C x240, D x135.

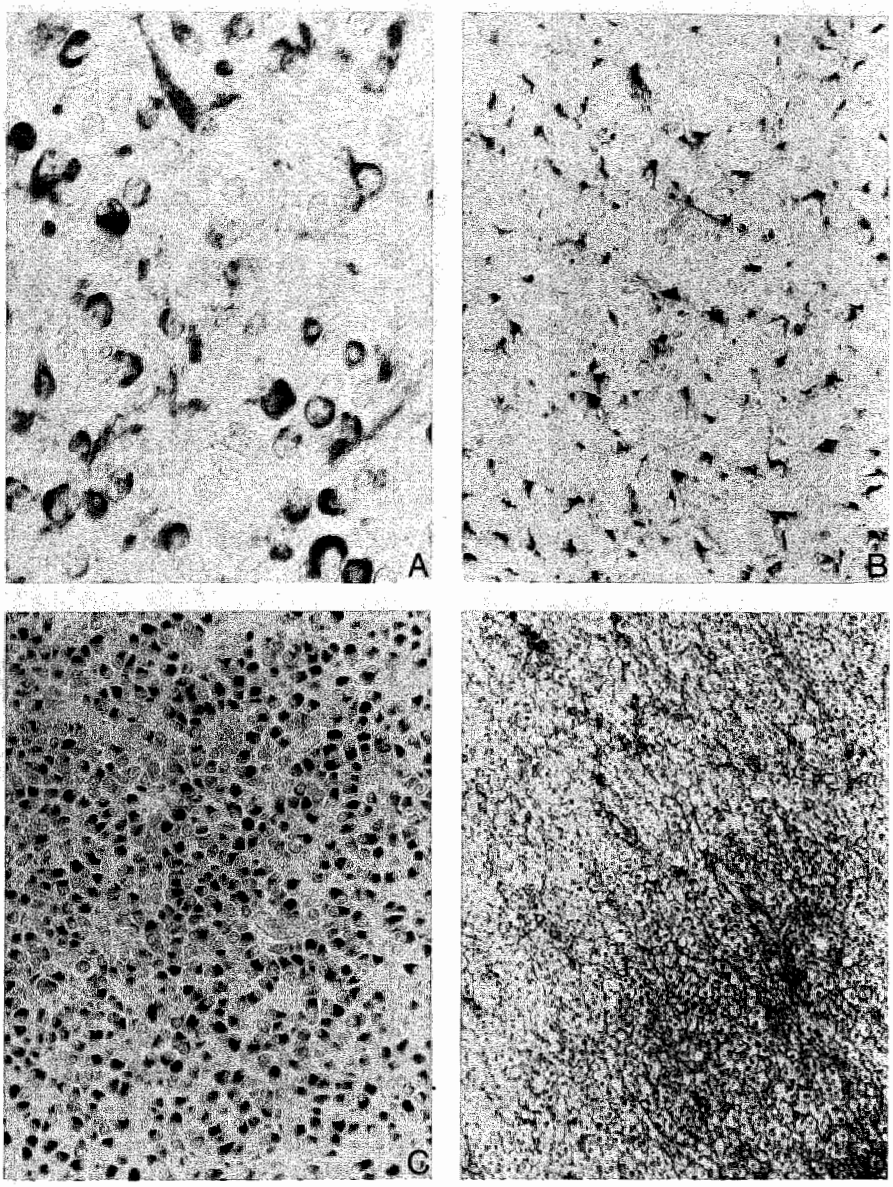


Fig. 2
A - C Different cases of transitional cell type tumor. A. Variability of GFAP-positive cell forms at a higher magnification; x600. B. Large cells similar to reactive astrocytes dominate the field although some small GFAP positive cells (gfocs) and slightly larger transitional cell forms are also seen; x216. C. Small gemistocytic cell forms are predominant and gfocs less frequent in a tumor ("minigemistocytoma") of honey-comb texture and many GFAP-negative cells; x216. D. Another type of GFAP immunoreactivity in many oligodendrogliomas. A dense network of cell processes surrounds GFAP-negative oligodendroglial tumor cells; x135.

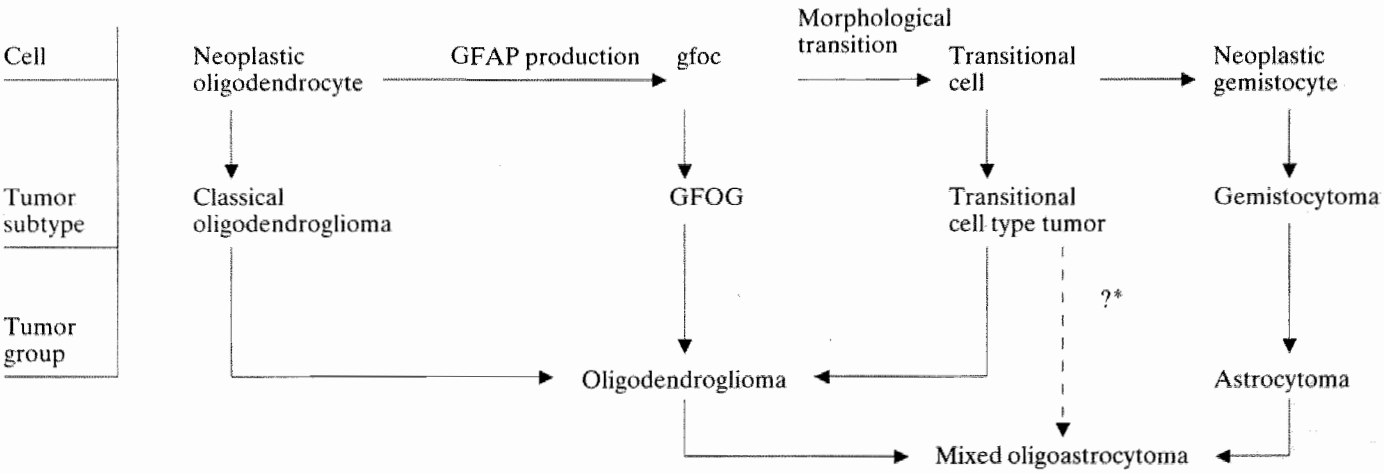
The astroglial theory seems unlikely in the light of the cytological appearance of gfocs. In addition, the primarily oligodendroglial character of such tumors was confirmed by electron microscopy as mentioned by Ishida et al. (1982).
With regard to the precursor cell hypothesis, the few recurrent tumors of this series did not show marked changes in the cellular composition which might be expected during evolution of a tumor developing from a precursor cell. However, strong support for the existence of a precursor or transitional cell form are the recent investigations by Choi and Kim (1983), reporting strong GFAP immunoreactivity in the cytoplasm and processes of cells with oligodendroglial morphology and associated with the development of compact myelin in the human spinal cord. Using EM, they found glial filaments in the cytoplasm of cells which were otherwise characteristic of oligodendroglia, and interpreted the GFAP positive cells as transitional cell forms. In vitro studies of Raff et al. (1983) also indicate the existence of a bipotentional progenitor cell. However, their progenitor cells were GFAP negative. Thus, the hypothesis of GFAP positive oligodendroglioma as a precursor cell neoplasm has still to be substantiated.

Transitional cell forms between oligodendroglial and astroglial tumor cells have been postulated by the use of classical neuro-histological silver impregnations. Progressive changes from neoplastic oligodendrocytes to astrocytes have been claimed to occur in vitro (Lumsden, 1971) and cytoplasmic filaments have been described to develop in oligodendrocytes in some experimental procedures (Bunge et al., 1961; Hirano and Zimmerman, 1971; Lampert et al, 1964). In experimental fibrillogenesis in oligodendroglia, filaments were indistinguishable ultrastructurally from those normally found in astrocytes (Hirano and Zimmerman, 1971). It is known, however, that filament formation in vitro is, in some instances, a non-specific phenomenon resulting from the structural adaptation of cells to the culture environment (Buckley and Porter, 1967; Lewis and Lewis, 1924; Weinstein and Kornblith, 1971). Ishida et al. (1982) noted a varying number of oligodendroglial cells with perikaryal staining for GFAP to which, at the ultrastructural level, bundles of glial filaments appeared to correspond. Meneses et al. (1982) and Kepes and Meneses (1982) described GFAP positive neoplastic oligodendroglial cells in 15 of 19 oligodendrogliomas and suggested that mixed oligoastrocytomas were actually tumors of a single cell type capable of differentiating along astrocytic lines.
The existence of a tumor cell combining morphological characteristics of oligodendroglia with GFAP production in its cytoplasm is strikingly reminiscent of the behaviour of normal myelin-forming glia during development with transient expression of GFAP (Choi and Kim, 1984). The latter finding in normal fetal development suggests two possibilities of interpretation: first, radial glia or radial glial cell-derived astroglia may transform into myelin-forming oligodendroglia or, second, cells committed to become oligodendroglia transiently express GFAP (Choi and Kim, 1984). Either possibility may account for GFAP production in neoplastic oligodendrocytes: a likely return to fetal behaviour by the cells of oligodendrogliomas. It must be admitted, however, that comparison of a neoplastic condition to normal fetal development is limited: the potential for phenotypic variation is often wider in neoplastic than in normal developing cells. In a neoplastic situation cells may exhibit features that can be

Table 3.
Summary of previous studies of oligodendrogliomas with anti-GFAP sera and the interpretation of immunoreactive tumor cells by various investigators.

Authors	No. of cases examined	GFAP immuno-reactivity	Interpretation
Conley (1979)	2, experimental in mice	All negative	–
DeArmond et al. (1980)	No data given	Unexpected number of cells may be positive	Unusual type of gemistocytic astrocyte, PTAH-negative
Deck et al. (1978)	No data given	All negative	–
Delpech et al. (1978)	3	All negative (immunochemical assay)	–
Eng and Rubinstein (1978)	No data given	All negative	–
Rasmussen et al. (1980)	5	Similar to astrocytomas and glioblastoma (quantitative immuno-electrophoresis)	–
Tascos et al. (1982)	3 Oligodendrogliomas 1 Oligoblastoma	All negative	–
Velasco et al. (1980)	3	All negative	–
Girard et al. (1983)	11	Positive in 6 cases	"Transdifferentiation" towards astrocytes
Ishida et al. (1982)	11	Varying number of cells positive	–
Meneses et al. (1982), and Kepes and Meneses (1982)	19	Positive in 15 cases	Single cell type able to differentiate along astrocytic lines: origin of mixed oligoastrocytoma
Van der Meulen et al. (1978)	18	Grade II: alle negative Grade III: positive in 3 of 13 cases Grade IV: all negative	Transition from oligodendrocytic tumor cells to astrocytic tumor cells in malignant oligodendroglioma

Table 4.
Possible interrelation of different GFAP immuno-reactive tumor cells found in oligodendroglial tumors and their relation to the respective tumor types.



?* = development of a mixed oligoastrocytoma from a transitional cell type tumor is conceivable but not supported by this study.

found only in normal or developing cells that are related to the cells of origin of the tumor.
Girard et al. (1983) found GFAP reactivity in 6 of 11 oligodendroglial tumors (a proportion very similar to that of our series) and related the small number of GFAP positive oligodendrogliomas described in literature to the fact that often only a few cells are labelled, thus creating a sampling problem. This is also suggested by the present study.

Tumors which show gfocs as a predominant cell population (three of 50 oligodendrogliomas of this series) apparently represent a special subtype of oligodendroglioma (gliofibrillary oligodendroglioma, GFOG) which is different from mixed oligoastrocytoma. Such a distinction is also supported by the fact that we found gfocs mainly in pure oligodendrogliomas and not in mixed oligoastrocytomas.
If mixed oligoastrocytomas are interpreted as tumors of a single cell precursor capable of differentiating along separate lines according to Raff et al. (1983), most gfocs were to be expected in mixed tumors. Our results, however, do not support this assumption. Thirty-two percent of all tumors showed areas with a gradual morphological change from gfoc to gemistocytic astroglial cells. This morphological spectrum exemplifies the potential of transition from classical oligodendrocytes to oligodendrocytes with an additional biochemical marker (GFAP), to cells developing further morphological characteristics of astroglial cells (dominating minigemistocytes in one case). Such transitional tumors also should be distinguished from oligoastrocytomas. It is conceivable, however, that mixed oligoastrocytomas may represent the end stages of the transition process which is caught by the neuropathologist's eye only at certain stages in the evolution of an oligodendroglioma. Table 4 schematises the possible relation of gfocs and transitional tumor cell forms to tumor subtypes and groups. The natural history of the oligodendroglioma subtypes described here (GFOG and transitional cell type oligodendroglioma or transitional oligoastrocytoma) should be defined by future studies. It remains to be seen if these subtypes gain clinical significance.

Acknowledgements
Excellent technical assistance was given by Ms. B. Windsperger.

References

1. **Buckley, I.K., Porter, K.R. (1967):**
   Cytoplasmic fibrils in living culture cells.
   Protoplasma 64: 349-380.
2. **Bunge, M.B., Bunge, R.P., Ris, H. (1961):**
   Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord.
   J. Biophys. Biochem. Cytol. 10: 67-94.
3. **Choi, B.H., Kim, R.C. (1983):**
   Immature oligodendroglial cells in developing human fetal spinal cord contain immunoreactive glial fibrillary acidic protein (GFAP).
   J. Neuropathol. Exp. Neurol. 42: 325 [Abstr.].
4. **Choi, B.H., Kim, R.C. (1984):**
   Expression of glial fibrillary acidic protein in immature oligodendroglia.
   Science 223: 497-509.
5. **Conley, F.K. (1979):**
   The immunocytochemical localization of GFA protein in experimental murine CNS tumors.
   Acta Neuropathol. (Berl) 45: 9-16.
6. **DeArmond S.J., Eng, L.F., Rubinstein, L.J. (1980):**
   The application of glial fibrillary acidic protein immunohistochemistry in neurooncology.
   Pathol. Res. Pract. 168: 374-394.
7. **Deck, J.H., Eng, L.F., Bigbee, J., Woodcock, S.M. (1978):**
   The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors.
   Acta Neuropathol. (Berl) 42: 183-190.
8. **Delpech, B., Delpech, A., Vidard, M.N., Girard, N., Tayot, J., Clement J.C., Creissard, P. (1978):**
   Glial fibrillary acidic protein in tumors of the nervous system.
   Brit. J. Cancer 37: 33-40.
9. **Eng, L.F., Rubinstein, L.J. (1978):**
   Contribution of immunochemistry to diagnostic problems of human cerebral tumors.
   J. Histochem. Cytochem. 26: 513-522.
10. **Girard, U., Courel, M.N., Delpech, B., Tayot, J., Creissard, P. (1983):**
    Two markers for brain tumors: Glial fibrillary acidic protein (GFAP) and Hyaluronectin. In: Peeters, H. (ed) Protides of the biological fluids. Proc. 30th Coll. 1982. Pergamon Press, Oxford, pp. 137-140.
11. **Herpers, M.J.H.M., Budka, H., McCormick, D. (1984):**
    Production of glial fibrillary acidic protein (GFAP) by neoplastic cells: adaptation to the microenvironment.
    Acta Neuropathol. (Berl) 64: 333-338.
12. **Hirano, A., Zimmerman, H.M. (1971):**
    Glial filaments in the myelin sheath after vinblastine implantation.
    J. Neuropathol. Exp. Neurol. 30: 63-81.

13. **Ishida, Y., Takahashi, K., Nakazato, Y. (1982):**
Immunohistochemical and electron-microscopic studies of experimental and human oligodendrogliomas. 11th Int. Congr. Neuropathol., Vienna, Sept. 1982, p 181 [Abstr. I-42].
14. **Kepes, J.J., Meneses, C.O.A. (1982):**
Astrocytic differentiation of neoplastic oligodendrocytes. 11th Int. Congr. Neuropathol., Vienna,
Sept. 1982, p 158 [Abstr. D5-11].
15. **Kernohan, J.W., Mabon, R.F., Svien, H.J., Adson A.W. (1949):**
Simplified classification of gliomas.
Proc. Mayo Clin. 24: 71-75.
16. **Kernohan, J.W., Sayre, G.P. (1952):**
Tumors of the central nervous system.
Armed Forces Institute of Pathology. Washington, DC.
17. **Lampert, P., Blumberg, J.M., Pentschew, A. (1964):**
An electronmicroscopic study of dystrophic axons in the gracile and cuneate nuclei of vitamin E-deficient rats.
J. Neuropathol. Exp. Neurol. 23: 60-77.
18. **Lewis, W.H., Lewis, M.R. (1924):**
Behaviour of cells in tissue culture. In: Cowdry, E.V. (ed) General cytology. University of Chicago Press, Chicago, pp 385-447.
19. **Lumsden, C.E. (1971):**
The study by tissue culture of tumors of the nervous system. In: Russell, D.S., Rubinstein, L.J. (eds) Pathology of tumors in the central nervous system, 3rd edn. Edward Arnold, London, pp 334-420.
20. **Meneses, A.C.O., Kepes, J.J., Sternberger, N.H. (1982):**
Astrocytic differentiation of neoplastic oligodendrocytes.
J. Neuropathol. Exp. Neurol. 41: 368 [Abstr.].
21. **Raff, M.C., Miller, R.H., Noble, M. (1983):**
A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium.
Nature 303: 390-396.
22. **Rasmussen, S., Bock, E., Warecka, K., Althage, G. (1980):**
Quantitation of glial fibrillary acidic protein in human brain tumors.
Brit. J. Cancer 41: 113-116.
23. **Rubinstein, L.J. (1972):**
Tumors of the central nervous system. In: Atlas of tumor pathology, ser. 2, fasc. 6, Armed Forces Institute of Pathology, Washington, D.C.
24. **Sternberger, L.A. (1979):**
Immunocytochemistry. 2nd edn. Wiley and Sons, New York Chichester Brisbane Toronto.
25. **Tascos, N.A., Parr, J., Gonatas, N.K. (1982):**
Immunocytochemical study of the glial fibrillary acidic protein in human neoplasms of the central nervous system.
Hum. Pathol. 13: 454-458.
26. **Van der Meulen, J.D.M., Houthoff, H.J. and Ebels, E.J. (1978):**
Glial fibrillary acidic protein in human gliomas.
Neuropathol. Appl. Neurobiol. 4: 177-190.

27. **Velasco, M.E., Dahl, D., Roessmann, U., Gambetti, P. (1980):**
Immunohistochemical localisation of glial fibrillary acidic protein in human glial neoplasms.
Cancer 45: 484-494.
28. **Weinstein, R.S., Kornblith, P.L. (1971):**
Ultrastructure of cloned astrocytoma in tissue culture.
Cancer 27: 1174-1181.
29. **Zülch, K.J. (1979):**
Histological typing of tumors of the central nervous system. International histological classification of tumors, no 21. WGO, Geneva.

PRIMITIVE NEUROECTODERMAL TUMORS INCLUDING THE MEDULLOBLASTOMA: GLIAL DIFFERENTIATION SIGNALED BY IMMUNOREACTIVITY FOR GFAP IS RESTRICTED TO THE PURE DESMOPLASTIC MEDULLOBLASTOMA ("ARACHNOIDAL SARCOMA OF THE CEREBELLUM")

Clin. Neuropathol. (1985) 4: 12-18.

Chapter 7

Primitive neuroectodermal tumors including the medulloblastoma: glial differentiation signaled by immunoreactivity for GFAP is restricted to the pure desmoplastic medulloblastoma ("arachnoidal sarcoma of the cerebellum").

M.J.H.M. Herpers, and H. Budka.

Abstract
Immunoreactivity of tumor cells for glial fibrillary acidic protein (GFAP) is usually regarded as sign of astrocytic histogenesis and/or differentiation. The present study aimed at a systematic evaluation of the significance of GFAP containing cells in primitive neuroectodermal tumors (PNETs) with special reference to the controversial entity of desmoplastic medulloblastoma (so-called "circumscribed arachnoidal sarcoma of the cerebellum"). Fifty-three PNETs, including 17 pure desmoplastic medulloblastomas were investigated, using GFAP antisera and the peroxidase-antiperoxidase (PAP) technique. Seventy percent of the pure desmoplastic medulloblastomas showed GFAP immunoreactive cells, in 47% indistinghuishable from adjacent nonreacting tumor cells. Most immunoreacting cells were found in the reticulin free islands, showing in 6 cases a gradual transition of immunoreacting cells from tumor cells to larger cells shaped like astrocytes. The classical medulloblastomas showed only larger immunoreacting cells which were interpreted as reactive astrocytes. Therefore, the so-called circumscribed arachnoidal cerebellar sarcoma or pure desmoplastic medulloblastoma merits a separate place in the group of PNETs as a tumor with frequent signs of astroglial differentiation; this interpretation appears to be clinically correllated by a difference in age incidence and prognosis of that special tumor-type in comparison with classical medulloblastoma.

Key-words
astroglial differentiation - desmoplastic medulloblastoma - GFAP - medulloblastoma - primitive neuroectodermal tumor - immunocytochemistry.

Introduction
Glial fibrillary acidic protein (GFAP) has been isolated as a specific constituent protein of astroglial intermediate filaments. The protein is easily detectable with immunohistochemical methods and has therefore been used as a specific marker for studies of glial cytology in a normal and pathologically altered central nervous system, as well as in CNS neoplasms. Primitive neuroectodermal tumors (PNETs), especially the classical medulloblastoma, have been studied immunohistochemically with special regard to diagnostic relevance and differentiation along glial lines using antisera to GFAP (Mannoji et al., 1981;

Palmer et al., 1981; Pasquier et al., 1983; Schindler and Gullotta, 1983). Among medulloblastoma, the desmoplastic variant (Rubinstein and Northfield, 1964) or so-called circumscribed arachnoidal sarcoma of the cerebellum (Foerster and Gagel, 1938) is a primary malignant posterior fossa tumor, usually found in young adults, most frequently located superficially in the lateral cerebellar lobes. Some features appear to distinguish this tumor from the classical medulloblastoma; usually the former features a large amount of reticulin fibers in a characteristic pattern and has been claimed to carry a better prognosis when completely extirpated (Spitz et al., 1947; Chatty and Earle, 1971). Its histogenesis has been debated for decades. Some authorities regard it as special features in a classical medulloblastoma when invading the leptomeninges (Rubinstein, 1970; Russell and Rubinstein, 1977), while others are more in favor of an at least partial sarcomatous nature (Kersting, 1967; Gullota, 1967[a] and 1981). This study aims at a systematic evaluation of GFAP-containing cells in PNETs with special reference to the desmoplastic medulloblastoma in which only few data on GFAP immunoreactivity have been reported so far.

Materials and methods

Fifty-three tumors belonging to the group of PNETs (Rorke, 1983) were studied. Table 1 summarizes diagnoses and number of cases studied.

All tumors were neurosurgical biopsies from the neuropathological collection of the Neurological Institute of the University of Vienna and classified according to the WHO classification (Zülch, 1979). The tumor specimens were fixed in 10% neutrally buffered formalin for an average of 24-36 hours and embedded and stored in paraffin. Prolonged storage did not alter immunoreactivity; storage time ranged from a few days up to 14 years.

Immunostained sections were compared to hematoxylin-eosin, Gomori, PTAH, and Bodian stains. The immunostained slides were selected from representative areas of the tumor and in most instances covered an area of several square centimeters.

The series of 18 classical medulloblastomas in 14 patients included 4 recurrencies; in two cases, cerebral spinal fluid (CSF) metastases were studied. According to the WHO blue book (Zülch, 1979), a diagnosis of desmoplastic medulloblastoma was made when silver impregnation stains demonstrated a prominent reticulin network. Among these tumors, two groups were distinguished. The first group included otherwise classical medulloblastomas strongly invading the leptomeninges in some parts, initiating a vigorous desmoplastic reaction. The second group included so-called circumscribed arachnoidal cerebellar sarcoma, diagnosed by a characteristic mosaic pattern of cell-dense and reticulin-rich whorled cell strains, surrounding less dense ('pale') reticulin free islands. This latter type was found in a total of 17 tumors in 12 patients including three recurrencies and one recurring CSF metastasis. Most tumors were found in patients between 20 and 51 years of age. However, 3 cases occurred in infants younger than 2 years old.

The peroxidase antiperoxidase (PAP) technique according to Sternberger (1979) was used.

The anti-GFAP serum was produced in rabbit and kindly provided by Dr. D. McCormick, Belfast, Nothern Ireland, and is described in more detail elsewhere (Herpers et al., 1984). Commercially available linking antibody (swine anti

rabbit IgG) and PAP-complex (rabbit-PAP) were purchased from DAKO, Copenhagen, Denmark, and normal swine serum from Gibco Europe, Glasgow, United Kingdom.

The optimal dilution of the primary antiserum (1:100) was empirically defined for the incubation time used of 30-45 minutes.
Specificity controls included substitution of the primary antiserum by nonimmune rabbit serum or in some cases by the anti-GFAP serum which had been preabsorbed with purified GFAP. All these control sections showed no immunoreactivity.
Sections containing a cerebral metastasis of a primary extracranial carcinoma, surrounded by reactive gliosis were used as staining controls.
Counterstaining with hemalum was optional and used for a more precise localization of the diaminobenzidine (DAB) reaction product.

Results

Table 1 summarizes the incidence of cells immunoreactive for GFAP.

Pinealoblastoma

Very few GFAP positive cells were seen. All were characterized by their spider appearance as reactive astrocytes.

Neuroblastoma

All three supratentorial cases were almost completely GFAP negative; focally a GFAP positive fibrillary network was found, apparently representing stromal astroglial processes. The two intraspinal extensions of retroperitoneal neuroblastoma were free of GFAP immunoreactive cells.

Classical medulloblastoma

In most examples, GFAP immunoreactive cells were absent in the bulk of the tumor. In three cases, focally large astrocytes and a perivascular network of cell processes were immunostained. Four other tumors showed focally an intensely immunostained fibrillar GFAP positive network comparable to the network of astroglial processes in many oligodendrogliomas stained for GFAP (Velasco et al., 1980; Herpers and Budka, 1984). In 15 cases, GFAP-immunostained cells showed a spider appearance and were found close to adjacent infiltrated brain parenchym. They were therefore interpreted as reactive astrocytes of preexistent CNS tissue (Fig. 1A). Such cells were included in Table 1 within the large cell category.

Desmoplastic medulloblastoma, classical variety invading the leptomeninges (desmoplastic reaction)

Eleven of 12 tumors of this group were negative or showed GFAP-immunostained structures which clearly belonged to preexisting parenchyma (9 cases).
One tumor showed focally GFAP-positive cells shaped like fibrillary astocytes, remote from surrounding brain tissue in areas with a fibrillary network of GFAP positive cell processes. These areas suggested spongioblastic differentiation in routine preparations and were absent in leptomeningeal extensions (Figs. 1B and C).

Table 1
Number of cases, classification of PNETs and incidence of immunoreactive cells.

Number of cases	Diagnosis	Median age ±SD (years)	Age range (years)	Number of cases with GFAP positive cells		
				Large cells indistinguishable from reactive astrocytes	Transitional cell forms	Small cells indistinguishable from tumor cells
1	Pinealoblastoma*	2	–	1	–	–
3	Primary supratentorial neuroblastoma**	16.6	10-28	1	–	–
2	Spinal extradural-extension of retroperitoneal neuroblastoma	2.7	2.5-3	–	–	–
18	Classical medulloblastoma	10.4±6.2	0.5-21	18	–	–
29	Desmoplastic medulloblastoma					
	12 Classical medulloblastoma clearly invading the leptomeninges (desmoplastic reaction)	13.0±10.9	1.7-33	9	–	–
	17 So-called circumscribed arachnoidal cerebellar sarcomas.	26.5±16.0	0.7-51	9	6	8
Total: 53						

* PNET of the pineal region also featuring tubular structures as described by Herrick and Rubinstein (1978) (case 4)
** Diagnosis confirmed by electron microscopy

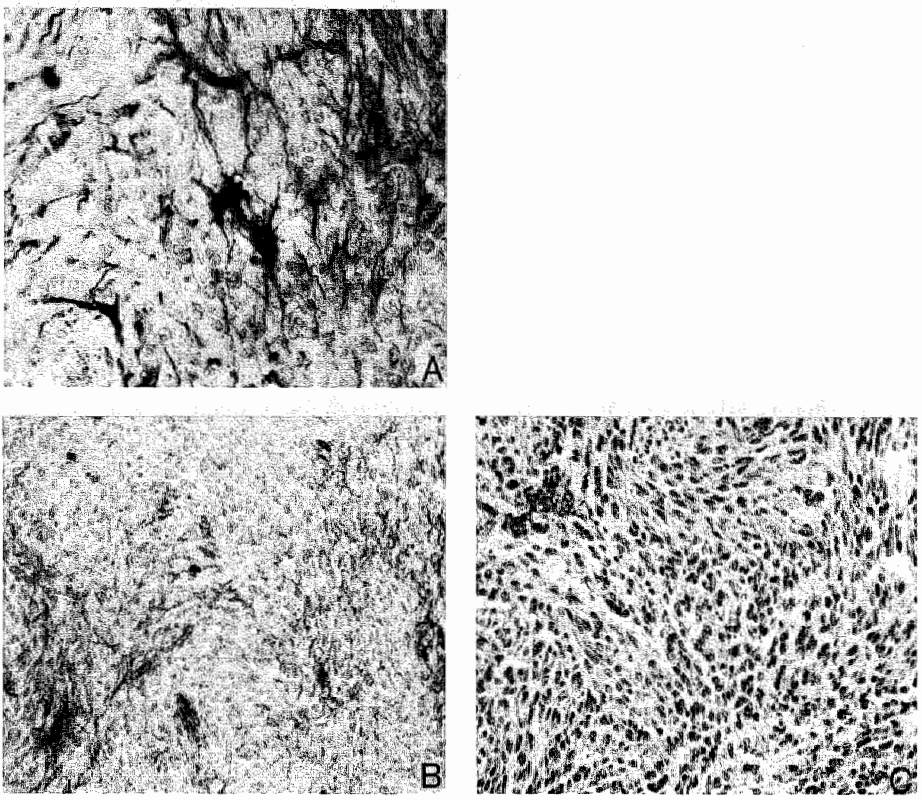


Fig.1
GFAP in classical medulloblastoma.
A. Large immunoreactive cells and their processes identifiable by their spider appearance as reactive astrocytes (GFAP-PAP, hemalum counterstain, x230).
B, C. Fine meshwork of GFAP positive cell processes and few larger cell bodies (B) in a medulloblastoma suggesting "spongioblastic differentiation" already in a routine hematoxylin-eosin preparation (C) (B and C x150).

Pure desmoplastic medulloblastoma, so-called circumscribed arachnoidal cerebellar sarcoma.
Twelve cases, including the recurrencies and the CSF metastases, showed focally GFAP positive cells, most frequently within the reticulin-free islands (Figs. 2A and B). Of these 12 cases, 8 tumors, including one recurrency and the CSF metastases, showed small cells immunoreactive for GFAP, which were indistinguishable from adjacent tumor cells and featured GFAP immunoreactivity in a narrow cytoplasmic rim, surrounding a hyperchromatic nucleus (Fig. 2D). These cells were most frequently located in the pale reticulin-free islands and apparently were equivalent to the type 1 cells of Mannoji et al. (1981). In two cases, small GFAP positive tumor cells were also seen in the more closely packed reticulin-rich areas (Fig. 2C) surrounding the pale islands. Nine tumors contained relatively large GFAP positive cells with prominent formation of processes which were indistinguishable from reactive astrocytes (comparable to type 3 cells of Mannoji et al. (1981). Such cells intermingled focally with

tumor cells and in two cases were prominently located at the periphery of pale islands areas (Fig. 2C). In 6 tumors, GFAP positive cells were found suggesting a gradual transition from small tumor cells to cells resembling reactive astrocytes by their appearance with a gradually broader immunoreactive cytoplasmic body (presumably equivalent to the type 2 cells of Mannoji et al. (1981) (Fig. 2E)). In PTAH-stained sections, alle areas containing small GFAP immunoreactive tumor cells remained unstained.

Discussion

Since Bailey and Cushing (1925) introduced the concept of the cerebellar medulloblastoma as a tumor composed of indifferent cells which are analogous to those of the developing cerebellum and potentially neuroglial, the problem of histogenesis and differentiation of this tumor has remained unsolved. Most investigators today regard the medulloblastoma as an embryonal neuroectodermal tumor with variable (glial and neuronal) differentiation potential (surveys by Rubinstein (1974) and Rorke (1983)). The medulloblastoma was studied immunohistochemically with GFAP antisera by several groups (Jacque et al., 1978; Van der Meulen et al., 1978; Deck et al., 1978; Delpech et al., 1978; Eng and Rubinstein, 1978; Herman and Rubinstein, 1978; Barnard and Pambekian, 1980; Velasco et al., 1980; Mannoji et al., 1981; Palmer et al., 1981; Tascos et al., 1982; Girard et al., 1983; Pasquier et al., 1983; Roessmann et al. 1983; Schindler and Gullotta, 1983; Coffin et al., 1983) whose results are summarized in Table 2.

Cells immunoreacting for GFAP and interpreted as tumor cells were generally regarded as sign of astrocytic differentiation. Coffin et al. (1983) and Schindler and Gullotta (1983) disagreed and declared immunohistochemical studies inadequate to give such interpretation. Despite some variability in results and interpretation, the other reports supported a neuroectodermal origin and glial differentiation potential of medulloblastomas. Recently, Rorke (1983) proposed a new classification scheme for primitive CNS tumors of infancy and childhood, including the medulloblastomas, based on their differentiation markers by the combined use of classical neurohistologic stains, electron microscopy and immunohistochemistry for GFAP and neurofilament proteins.

Previous immunohistochemical studies included only very few if any medulloblastomas of the desmoplastic variant. Only Schindler and Gullotta (1983) included 29 examples in their study (classified by them as desmoplastic medulloblastoma or as mixed neuroectodermal-mesodermal tumor) and found GFAP positive cells in four of them, a lesser incidence than in the present study. However, there is now enough evidence for the very existence of GFAP-containing medulloblastoma cells which are mainly restricted to the desmoplastic variety. Pseudopositivity of these tumor cells due to protein diffusion or phagocytosis (Schindler and Gullotta, 1983) is most unlikely since classical medulloblastomas did now show GFAP positive tumor cells even in close proximity to reactive astrocytes very rich in GFAP.

Regardless of interpretation of the desmoplastic variant as a extracerebellar growth pattern of medulloblastoma (Rubinstein and Northfield, 1964; Rubinstein, 1970) or as mixed mesenchymal-neuroectodermal tumor (Gullotta, 1967a and b), glial differentiation should be traceable by immunohistochemistry for GFAP in the same way as in the classical medulloblastoma of the midline (Table 2).

Table 2
Results of GFAP immunostaining studies of medulloblastomas by various groups.

Author(s)	CM or not otherwise specified, No. of cases showing GFAP reactivity	DM No. of cases showing GFAP reactivity	Remarks by the respective invesigators
Deck et al. (1978)	no data	1	greater number of reacting cells in reticulin free islands
Eng and Rubinstein (1978)	some cases	–	astrocytic origin of differentiation
Van der Meulen (1978)	6: all negative	–	even areas suggesting astrocytic differentiation were GFAP negative
Velasco et al. (1980)	3: some positive	–	areas of astrocytic differentiation
Mannoji et al. (1981)	24: 3 showing GFAP reactive type 1 cells	1: positive in pale islands	glial character is expressed more within the pale islands
Palmer et al. (1981)	13: 11 GFAP positive	–	astroglial differentiation
Tascos et al. (1982)	3: all negative	–	–
Girard et al. (1983)	3: 2 containing GFAP positive cells	–	–
Pasquier et al. (1983)	17: 11 with type 1 cell	1: type 1 in pale islands	astrocytic differentiation
Roessmann et al. (1983)	47: 23 GFAP positive	–	–
Schindler and Gullotta (1983)	21: 5 with GFAP positive tumor cells	29: 4 with positive tumor cells	'pseudopositivity' (stromal astrocytes) in 28 of 50 cases
Coffin et al. (1983)	17: 3 GFAP positive	3: 2 with positive cells	GFAP positivity may not be intrinsic to medulloblastoma and must be interpreted with skepticism
Present series	18: only large reactive astrocytes	17: 8 with immunoreactive tumor cells	glial differentiation mainly in pale islands

CM = Classical Medulloblastoma; DM = desmoplastic variant and so-called arachnoidal cerebellar sarcoma.

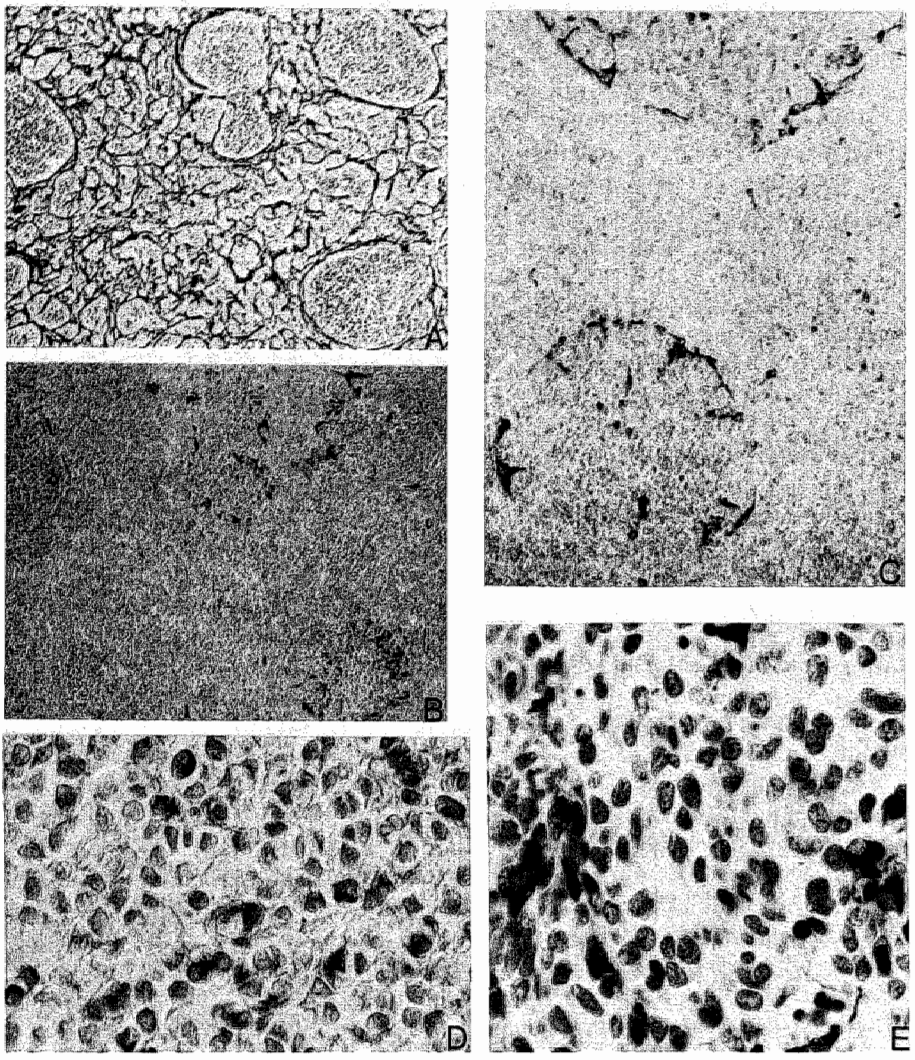


Fig. 2
GFAP in pure desmoplastic medulloblastoma ("arachnoidal sarcoma of the cerebellum").
A, B. Characteristic pattern in reticulin preparations (A) with reticulin-rich areas surrounding pale islands; most GFAP positive cells are situated within the islands in an adjacent section (B) (A and B x63, A. Gomori stain, B. GFAP-PAP, hemalum counterstain).
C. Large immunolabeled cells are situated mainly at the periphery of the islands; small GFAP positive cells prefer a more central site within the islands and, in lesser frequency, in the surrounding tissue (GFAP-PAP, slight hemalum counterstain, x150).
D. Small cells with narrow GFAP positive cytoplasmic rim are scattered among tumor cells with otherwise identical appearance (GFAP-PAP, slight hemalum counterstain, x600).
E. Variation of size of GFAP-containing tumor cell cytoplasm including small cells similar to D and larger forms more similar to astrocytes, suggesting transition from small to larger cell forms (GFAP-PAP, hemalum counterstain x 600).

The main problem, however, of such immunohistochemical studies is the appropriate interpretation of immunostained cells as neoplastic, reactive, stromal or preexisting, respectively, or still more intriguing, as a secondary phenomenon due to phagocytosis or protein diffusion (Schindler and Gullotta, 1983). Considering the limited resolution of the light microscope this problem can not always be solved when interpreting individual cells. However, by systematic evaluation of the immunostaining pattern in large tumor series, consistency of a given pattern in a given tumor type renders bias by erroneous interpretation of individual results less likely. In this study consistent results of GFAP immunoreactivity in PNETs were found only in one tumor group: the pure desmoplastic medulloblastoma, further stressing the separation of this variant from classical medulloblastoma.

The so-called circumscribed arachnoidal cerebellar sarcoma has therefore to be interpreted as a neuroectodermal tumor, or at least in major parts of neuroectodermal origin, with frequent signs of astroglial differentiation. In agreement with previous descriptions (Deck et al., 1978; Mannoji et al., 1981), most GFAP immunoreactive cells were found within the pale tumor islands. This is in accordance with Rubinstein and Northfield (1964), who noted the loss of a fibrillary neuroectodermal matrix outside of the pale islands. One may argue that the reticulin-free islands are preexistent tissue elements infiltrated by tumor cells. However, this is unlikely according to Rubinstein and Northfield (1964) who clearly stated that the pattern of islands in the desmoplastic medulloblastoma is invariably restricted to portions of the tumor spreading in the subarachnoid space. Furthermore the characteristic mosaic pattern of pale islands may be repeated in CSF and even extraneural metastases (Rubinstein and Northfield, 1964) and was also found in two metastases of the present series. Large astroglial immunoreactive cells were sometimes situated at the periphery of pale islands, i.e., immediately adjacent to surrounding reticulin strands. Their appearance in such location may be analogous to the glial cells bordering mesenchymal tissue which show an increase in immunoreactivity for GFAP (Herpers et al., 1984).These cells, although morphologically indistinguishable from reactive astrocytes, could represent the final point of glial differentiation of tumor cells. This is also suggested by the occurrence of apparently transitional cell forms in many cases. An interpretation of the large GFAP-containing elements at the periphery of pale tumor islands as non-tumerous reactive astrocytes is weakened by Rubinstein and Northfield's (1964) observation that pale tumor islands are restricted to the subarachnoid space where the development of prominent reactive gliosis is unlikely.
The 18 classical medulloblastomas and the group with desmoplastic reaction showed a lack of GFAP reactivity, stressing the undifferentiated character of this neoplasm. However, occasional small tumor areas with few GFAP positive tumor cells might indicate a glial/spongioblastic differentiation (Eng and Rubinstein, 1978; DeArmond et al., 1980; Eng and DeArmond, 1981 and 1982; Schindler and Gullotta, 1983) as also suggested by one case in the present series.

Results of GFAP immunostaining in PNETs may have clinical significance. The definition of the desmoplastic variant as a differentiating medulloblastoma, as suggested by this study*, appears well correlated to clinical data on the desmoplastic medulloblastoma which differ in age incidence and prognosis (Spitz et al., 1947; Chatty and Earle, 1971) from the classical medulloblastoma.

Acknowledgements

The authors are most indebted to Dr. D. McCormick, Belfast, Northern Ireland, for kindly providing the GFAP antiserum. Excellent technical assistance was given by Ms. Brigitte Windsperger.

* Since submission of this manuscript, many tumors of the present series were also immunostained for neuron-specific enolase (NSE), another differentiation marker of nervous and neuroendocrine systems. Immunoreactivity for NSE, among PNETs, was usually restricted to the pale islands of pure desmoplastic medulloblastomas. This is further support for the frequent differentiation of the desmoplastic medulloblastoma.

References

1. **Bailey, P., Cushing, H. (1925):**
   Medulloblastoma cerebelli. A common type of midcerebellar glioma of childhood.
   Arch. Neurol. Psychiatry 14: 192-224.
2. **Barnard, R.O., Pambekian, H. (1980):**
   Astrocytic differentiation in medulloblastoma.
   J. Neurol. Neurosurg. Psych. 43: 1041.
3. **Chatty, E.M., Earle, K.M. (1971):**
   Medulloblastoma. A report of 201 cases with emphasis on the relationship of histologic variants to survival.
   Cancer 28: 977-983.
4. **Coffin, C.M., Mukai, K., Dehner, L.P. (1983):**
   Glial differentiation in medulloblastomas. Histogenetic insight, glial reaction, or invasion of brain?
   Am. J. Surg. Pathol. 7: 555-565.
5. **DeArmond S.J., Eng, L.F., Rubinstein, L.J. (1980):**
   The application of glial fibrillary acidic protein immunohistochemistry in neurooncology.
   Path. Res. Pract. 168: 374-394.
6. **Deck, J.H., Eng, L.F., Bigbee, J., Woodcock, S.M. (1978):**
   The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors.
   Acta Neuropathol. (Berlin) 42: 183-190.
7. **Delpech, B., Delpech, A., Vidard, M.N., Girard, N., Tayot, J., Clement, J., Creissard, P. (1978):**
   Glial fibrillary acidic protein in tumors of the nervous system.
   Br. J. Cancer 37: 33-40.
8. **Eng, L.F., Rubinstein, L.J. (1978):**
   Contribution of immunohistochemistry to diagnostic problems of human cerebral tumors.
   J. Histochem. Cytochem. 26: 513-522.
9. **Eng, L.F., DeArmond, S.J. (1981):**
   Glial fibrillary acidic protein immunocytochemistry in development and neuropathology. In: 11th Intern. Congr.
   Anat.: Glial and Neuronal Cell Biology, pp 65-79.
10. **Eng, L.F., DeArmond, S.J. (1982):**
    Immunocytochemical studies of astrocytes in normal development and disease.
    Adv. Cell. Neurobiol. 3: 145-171.
11. **Foerster, O., Gagel, O., (1938):**
    Das umschriebene Arachnoidalsarkom des Kleinhirns.
    Z. Ges. Neurol. Psych. 164: 565.
12. **Girard, N., Courel, M.N., Delpech, B., Tayot, J., Creissard, P. (1983):**
    Protides of the biological fluids. In: H. Peeters (ed) Proc. 30th Coll. 1982. Pergamon Press, Oxford, p 137-140.

13. **Gullotta, F. (1967[a]):**
Vergleichende Untersuchungen zur Morphologie und Genese der sogenannten Medulloblastome.
Acta Neuropathol. (Berlin) 8: 76-83.
14. **Gullotta, F. (1967[b]):**
Das sog. Medulloblastom. Springer, Berlin.
15. **Gullotta, F. (1981):**
Morphological and biological basis for the classification of brain tumors. With a comment on the WHO classification 1979: In: Krayenbühl, H. (ed) Advances and technical standards in neurosurgery, vol. 8, Springer, New York, pp 123-165.
16. **Herman, M.M., Rubinstein, L.J. (1978):**
Divergent glial and neuronal differentiation in a human medulloblastoma maintained in an organ culture system.
J. Neurop. Exp. Neurol. 37: 627.
17. **Herpers, M.J.H.M., Budka, H. (1984):**
Glial fibrillary acidic protein (GFAP) in oligodendroglial tumors: gliofibrillary oligodendroglioma and transitional oligoastrocytoma as subtypes of oligodendroglioma.
Acta Neuropathol. (Berlin) 64: 265-272.
18. **Herpers, M.J.H.M., Budka, H., McCormick, D. (1984):**
Production of glial fibrillary acidic protein (GFAP) by neoplastic cells: adaptation to the microenvironment.
Acta Neuropathol. 64: 333-338.
19. **Herrick, M.K., Rubinstein, L.J. (1979):**
The cytological differentiating potential of pineal parenchymal neoplasm (true pinealomas). A clinicopathological study of 28 tumors.
Brain 102: 289-320.
20. **Jacque, C.M. (1978):**
Determination of glial fibrillary acidic protein (GFAP) in human brain tumor.
J. Neurol. Sci. 35: 147-155.
21. **Kersting, G. (1967):**
Die Gewebszüchtung der Medulloblastome.
Acta Neuropathol. (Berlin) 8: 100-103.
22. **Mannoji, H. (1981):**
Glial fibrillary acidic protein in medulloblastoma.
Acta Neuropathol. 55: 63-69.
23. **Palmer, J.O., Kasselberg, A.G., Netsky, M.G. (1981):**
Differentiation of medulloblastoma.
J. Neurosurg. 55: 161-169.
24. **Pasquier, B., Lachard, A., Pasquier, D., Couderc, P., Delpech, B., Courel, M.N. (1983):**
Protéine gliofibrillaire acide (GFA) et tumeurs nerveuses centrales. Etude immunohistochimique d'une série de 107 cas. IIe Partie.
Ann. Pathol. 3: 203-211.

25. **Roessmann, U., Velasco, M.E., Gambetti, P., Autilio-Gambetti, L. (1983):**
Neuronal and astrocytic differentiation in human neuroepithelial neoplasm. An immunohistochemical study.
J. Neuropathol. Exp. Neurol. 42: 113-121.
26. **Rorke, L.B. (1983):**
The cerebellar medulloblastoma and its relationship to primitive neuroectodermal tumors.
J. Neuropathol. Exp. Neurol. 42: 1-15.
27. **Rubinstein, L.J. (1970):**
Tumors of the central nervous system. In: Atlas of tumor pathology, Series 2, Fascicle 6 Washington DC: Armed Forces Institute of Pathology.
28. **Rubinstein, L.J. (1974):**
The cerebellar medulloblastoma. Its origin, differentiation, morphological variants, and biological behavior. In: Vinken, P.J., Bruyn, G.W. (eds) Handbook of clinical neurology. Vol 18, pp 167-193 North-Holland, Amsterdam.
29. **Rubinstein, L.J., Northfield, D.W.C. (1964):**
The medulloblastoma and the so-called 'arachnoidal cerebellar sarcoma'. A critical reexamination of a nosological problem.
Brain 87: 379-412.
30. **Russell, D.S., Rubinstein, L.J. (1977):**
Pathology of tumors of the nervous system. 4th edn. Williams and Wilkins, Baltimore.
31. **Schindler, E., Gullotta, F. (1983):**
Glial fibrillary acidic protein in medulloblastomas and other embryonic CNS tumors of children.
Virchows Arch. (Pathol. Anat.) 398: 263-275.
32. **Spitz, E.B. et al. (1947):**
Cerebellar medulloblastoma in adults. Arch. Neurol. Psychiat. 57: 417-422.
33. **Sternberger, L.A. (1979):**
Immunocytochemistry, 2nd edn. John Wiley and Sons, New York.
34. **Tascos, N.A. et al. (1982):**
Immunocytochemical study of the glial fibrillary acidic protein in human neoplasms of the central nervous system.
Hum. Pathol. 13: 454-458.
35. **Van der Meulen, J.D.M., Houthoff, H.J., Ebels, E.J. (1978):**
Glial fibrillary acidic protein in human gliomas.
Neuropathol. Appl. Neurobiol. 4: 177-190.
36. **Velasco, M.E., Dahl, D., Roessmann, U., Gambetti, P. (1980):**
Immunohistochemical localization of glial fibrillary acidic protein in human glial neoplasms.
Cancer 45: 484-494.
37. **Zülch, K.J. (1979):**
Histological typing of tumors of the central nervous system. International Histological Classification of Tumors, No. 21, Geneva: WHO.

Chapter 8

Discussion

In neurooncology the application of modern methods of investigation has led to increased knowledge concerning the etiology, pathogenesis and histogenesis of central nervous system tumors.
Models of experimentally induced tumors have provided new insights with regard to the etiology and pathogenesis of cerebral neoplasms. Tissue culture studies of nearly all different tumors of the central nervous system have added much to our knowledge about the dynamic properties of the neoplastic cells and have shed new light on current concepts of the histogenesis of brain tumors with important implications for classification. The ultrastructural morphology of brain tumors has been elucidated by electron microscopy. Biochemical analysis in combination with immunocytochemical investigations has provided information about the intracellular structure and functional morphology of brain(tumor)cells, including the pattern of expression of intermediate filament proteins. In the present study the pattern of expression of intermediate filament proteins in neoplastic neuro-ectodermal cells in **qualitative** as in **quantitave** terms has been investigated in relation with problems of histogenesis, classification and grading of malignancy of these neoplasms.
The present investigations clearly show that intense GFAP immunoreactivity occurs in reactive non-neoplastic and highly differentiated astrocytes, but also in anaplastic astrocytomas.

In all the glioblastomas we investigated, many neoplastic cells were GFAP immunoreactive, supporting the astrocytic lineage of this tumor entity. In contrast Ringertz (1950 and 1964) considered that many primary malignant glioblastomas could not be derived from more highly differentiated astrocytomas. Likewise, Scherer (1940) distinguished two different types of glioblastoma, one arising the novo, and one the result of dedifferentiation of a preexisting more highly differentiated astrocytoma. Our observations on GFAP expression in these tumors make this distinction rather redundant.

Previous studies have shown that vimentin is a major constituent IF protein of immature glial cells, and occurs even before GFAP is expressed (Dahl et al., 1981a; Schnitzer et al., 1981; Shaw et al., 1981; Björklund et al., 1984). Vimentin, however, is only occasionally found, or even entirely absent in mature brain and spinal cord (Dahl et al., 1981b; Björklund et al., 1984). In rat glial tumor cell lines (Franke et al., 1978) human glioma cell lines (Franke et al., 1978; Paetau et al., 1979) and human gliomas in situ (Roessmann et al., 1983; Yung et al., 1985; present investigations) vimentin has been found to be consistently expressed.

Although vimentin expression seems to be initiated upon neoplastic transformation of astrocytes, no consistent changes in the class and quantity of IFP was found in progressively malignant (from grade I to grade IV) astrocytomas. Therefore, the expression of GFAP and vimentin is not related with the degree of anaplasia. The proposal of Trojanowski et al. (1984), which suggests that the ratio of GFAP positive to vimentin positive tumor cells within a given tumor specimen might provide objective criteria for grading and prognostic predictions, therefore now seems untenable.

An intriguing aspect of neoplastic growth is the phenomenon of intra- and intertumor cellular heterogeneity. In neurooncology an important aspect of cellular heterogeneity is the variable expression of GFAP and vimentin in the neoplastic cells. The most likely explanation for this observation is that in one tumor different cells may show different stages of differentiation. In principle for differentiational heterogeneity three mechanisms can be proposed. Firstly in a tumor individual neoplastic cells may have lost their capacity to differentiate to a variable extent (dedifferentiation). This explanation relies on the supposition that the initial transformations occured in a fully differentiated cell. A second possibility is that the initial transformation occurred in an immature pluripotential cell of which the neoplastic descendants have a variable capacity to differentiate. A third possibility might be that tumors with different patterns of expression of GFAP and vimentin might have been derived from glial cells of different cortical layers.

From a biological point of view the first explanation, dedifferentiation of mature transformed cells, seems rather unlikely. A developmental model of tumor differentiation, the second explanation, would be more in keeping with modern concepts of tumor biology.
This concept might provide an explanation for the heterogeneous expression of GFAP and vimentin in different cells in one tumor. It implies that glial "stem" cells persist in the mature CNS, and that neoplastic cells retain the differentiational capabilities of their non-neoplastic counterparts and thus might differentiate in an analogous way. The brain, however, is generally considered to posess at the most very few of these cells, possibly in the subependymal region. According to Rubinstein (1985) astrocytes in the G0 phase of the cell cycle are capable to reenter a proliferative state after the proper stimulus. Such a mechanism has also been proposed by Ludwin (1984) for the proliferation of oligodendrocytes, astrocytes and microglia, following a trauma to the CNS. The third possibility would also provide an explanation for the heterogeneity of GFAP or vimentin expression by glial tumor cells. Protoplasmic astrocytes, found in the middle layers of the cortex and the basal ganglia only contain very few or no glial filaments and consequently have been found to be GFAP-negative. On the other hand, astrocytes in the white matter and superficial and deep cortical layers, contain abundant intracytoplasmic filaments (Peters et al, 1978; Miller and Raff, 1984) and do show an intense immunostaining for GFAP. However, astrocytomas which arise "purely" in the hypothalamic region or in the brain-stem, are heterogeneous as well and show the above mentioned mixture of GFAP immunoreactive - and nonimmunoreactive tumor cells. Therefore this last hypothesis is not likely to be the true explanation.

Whatever the true explanation for the observed differentiational heterogeneity may be, it seems likely that this phenomenon has important implications for tumor progression. Selective loss of differentiation of transformed mature cells or of differentiational capacity of transformed immature cells during the course of development of the neoplasm may result in the selective proliferation of undifferentiated tumor cells with a more agressive biological behavior.

In chapter 5 we described enhancement of GFAP expression of neoplastic astrocytes located perivascularly, or invading mesenchymal tissues, which was explained as a micro-environmental adaptation. This phenomenon was not observed with regard to the expression of vimentin.
The abscence of accentuated expression of vimentin by perivascularly located tumor cells suggests that the GFAP and vimentin intermediate filament systems have different functions. This contention is supported by the differences we observed in the intracellular distribution of these IFP in astroglial (tumor)cells as described and discussed in chapter 6. These observations strongly suggest that two functionally different systems of IF in astroglia exist (Dahl et al., 1981a). This conclusion is in apparent contrast with the findings of Quinlan and Franke (1983), Sharp et al. (1982) and Wang et al. (1983), who described that GFAP and vimentin colocalize and even form heteropolymers. However, their observations concern IF systems in cultured glioma cells, and it cannot be excluded that the "artificial" tissue culture environment may influence the expression and distribution of the IF systems.

The immunohistochemical studies described in chapter 7 have led us to propose the existence of two histologically distinguishable types of oligodendroglioma, which may reflect the different developmental stages of oligodendroglial cells during normal development (Choi and Kim, 1984). Again, also in these cases the existence of a mixed population of tumor stem cells, reflecting different developmental stages of oligodendroglia, and subsequent proliferation of one subpopulation, or the loss of differentiational characteristics in the course of neoplastic development, might be responsible for the heterogeneous GFAP expression. Analogous to the astrocytomas, the possibility that these tumor cells go through a sequence of various developmental stages cannot be ruled out. The latter hypothesis seems to be supported by studies of vimentin expression in oliogodendrogliomas (13 cases) and mixed oligo-astrocytomas (18 cases) (unpublished data). In oligodendrogliomas, small GFAP positive neoplastic oligodendrocytes (gliofibrillary oligodendrocyte = gfoc) showed a pattern of vimentin immunoreactivity similar to that of GFAP whereas the larger GFAP positive "transitional cells" and "babygemistocytes" (bg) showed a significantly lower incidence of vimentin immunoreactivity. One oligodendroglioma almost completely consisted of gliofibrillary oligodendrocytic tumor cells, reacting identically with both antisera, whereas five tumors showed predominance of transitional cells which only reacted with GFAP anti-sera. In mixed oligo-astrocytomas the number of GFAP and vimentin immunoreactive tumor cells was much lower than in pure oligodendrogliomas. The observations on immunoreactivity for GFAP and vimentin in pure oligodendrogliomas contrasted with those of the mixed oligoastrocytomas, in which these proteins occurred with roughly equal frequencies in all different tumor cells (gfoc,

transitional cells, bg). Therefore, studies on vimentin support the separation of the two oligodendroglial subtypes from the group of mixed oligo-astrocytomas. In analogy to vimentin expression of immature glial cells (Dahl et al., 1981a; Schnitzer et al., 1981; Shaw et al., 1981; Björklund et al., 1984) where the expression of vimentin precedes that of GFAP, a similar developing sequence in IF content of neoplastic oligodendrocytes might occur. In contrast Schnitzer et al. (1981) and Yung et al. (1985) did not find vimentin reactivity within oligodendrocytes of developing and adult mouse CNS, and human oligodendrogliomas respectively.

Our immunohistochemical studies suggest that the desmoplastic variant of medulloblastoma (Rubinstein and Northfield, 1964) which has also been called "arachnoidal sarcoma of the cerebellum" (Foerster and Gagel, 1938) showing GFAP positive tumor cells is a differentiating medulloblastoma. Our findings strongly support the separation of this tumorgroup from other embryonal neuro-epithelial tumors, which sofar was only based on histological characteristics, different age-incidence and different prognosis. The different opinions concerning the validity of this subclass of medulloblastoma since the introduction of cerebellar medulloblastoma by Bailey and Cushing (1925), has been discussed extensively in chapter 8.

Ultrastructural and immunohistochemical investigations have shed new light on the controversial group of **embryonal central neuro-epithelial tumors** or **primitive neuroectodermal tumors (PNET).**
Rorke (1983) recently introduced a simplified classification system for undifferentiated neuroepithelial CNS neoplasms based upon the light microscopical appearence, intermediate filament immunocytochemistry and electron microscopy, but not including the localisation of the tumor. She proposed that primitive neuroectodermal cells persist at all levels of the CNS in the subependymal zone, which may be transformed and then give rise to undifferentiated tumors.
Rubinstein (1985) criticized this classification as an oversimplification, since it seems very unlikely that subependymal cells preserve the full spectrum of differentiation potencies during different stages of development. Our studies however support part of Rorke's hypothesis and are in agreement with the developmental concept of differentiation of glial neoplastic cells. Rorke's studies also illustrate that for the final classification of a morphologically undifferentiated CNS tumor, all available techniques should be applied.

In conclusion, immunohistochemical detection of intermediate filament proteins provides new information with regard to the histogenesis and biology of neuro-ectodermal brain tumors and adds new parameters for the classification of these neoplasms. Therefore, fundamental intermediate filament research is not only of cellbiological interest but may finally also have therapeutic consequences.

References

1. **Bailey, P. and Cushing, H. (1925):**
   Medulloblastoma cerebelli. A common type of midcerebellar glioma of childhood.
   Arch. Neurol. Psychiatry 14: 192-224.
2. **Bailey, P. and Cushing, H. (1926):**
   Tumors of the glioma group.
   J.B. Lippincott, Philadelphia.
3. **Björklund, H., Eriksdotter-Nilsson, M., Dahl, D. and Olson, L. (1984):**
   Astrocytes in smears of CNS tissues as visualized by GFA and Vimentin-immunofluorescence.
   Med. Biol. 62: 38-48.
4. **Choi, B.H. and Kim, R.C. (1984):**
   Expression of glial fibrillary acidic protein in immature oligodendroglia.
   Science 223: 497-509.
5. **Dahl, D., Rueger, D.C. Bignami, A., Weber, K. and Osborn, M. (1981[a]):**
   Vimentin, the 57.000 dalton protein of fibroblast filaments is the major cytoskeletal component in immature glia.
   Eur. J. Cell. Biol. 124: 191-196.
6. **Dahl, D. Bignami, A., Weber, K. and Osborn M. (1981b):**
   Filament proteins in rat optic nerves undergoing Wallerian degeneration. Localization of vimentin, the fibroblastic 100 Å filament protein, in normal and reactive astrocytes.
   Exp. Neurol. 73: 496-506.
7. **Foerster, O. and Gagel, O. (1938):**
   Das umschriebene Arachnoidalsarkom des Kleinhirns.
   Z. Ges. Neurol. Psych. 164: 565.
8. **Franke, W.W., Schmid, E., Osborn, M. and Weber, K. (1978):**
   Different intermediate-sized filaments distinguished by immunofluorescence microscopy.
   Proc. Natl. Acad. Sci. U.S.A. 75: 5034-5038.
9. **Goldman, R.D., Goldman, A.E., Green, K.J., Jones, J.C.R., Lieska, N., Talian, J.C., Yang, H.-Y. and Zackroff, R.V. (1984);**
   Intermediate filaments: their interactions with various cell organelles and their associated proteins.
   J. Submicrosc. Cytol. 16: 73-74.
10. **Ludwin. S.K. (1984):**
    Proliferation of mature oligodendrocytes after trauma to the central nervous system.
    Nature 308: 274-275.
11. **Miller, R.H. and Raff, M.C. (1984):**
    Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct.
    J. Neuro. Sci. 4: 585-592.

12. **Paetau, A., Virtanen, I., Stenman, S., Kurke, P., Linder, E., Valeri, Al, Westermark, B., Dahl, D. and Holtia, M. (1979):**
Glial fibrillary acidic protein and intermediate filaments in human glioma cells.
Acta Neuropathol. (Berl.) 47: 71-74.
13. **Peters, A., Palay, S.L. and Webster, H. de F. (1970):**
The fine structure of the nervous system. The cells and their processes. New York: Hoeber Medical Division, Harper and Row.
14. **Quinlan, R.A. and Franke W.W. (1983):**
Molecular interactions in intermediate filaments revealed by chemical cross-linking; Heteropolymers of vimentin and glial filament protein in cultured human glioma cells.
Eur. J. Biochem. 132: 477-484.
15. **Ringertz, U. (1950):**
Grading of gliomas.
Acta path. Scand., (Copenh.) 27: 51-64.
16. **Ringertz, U. (1964):**
In Zulch, K.J. and Woolf, A.L.: Classification of brain tumours.
Acta Neurochir., Suppl. 12.
17. **Roessmann, U., Velasco, M.E., Gambetti, P. and Antilio-Gambetti, L. (1983) (abstract):**
Vimentin intermediate filaments are increased in human neoplastic astrocytes.
J. Neuropathol. Exp. Neurol. 42: 309.
18. **Rorke, L.B. (1983):**
The cerebellar medulloblastoma and its relationship to primitive neuroectodermal tumors.
J. Neuropathol. Exp. Neurol. 42: 1-15.
19. **Rubinstein, L.J. and Northfield, D.W.C. (1964):**
The medulloblastoma and the so-called "arachnoidal cerebellar sarcoma". A critical reexamination of a nosological problem.
Brain 87: 397-412.
20. **Rubinstein, L.J. (1985):**
Embryonal central neuroepithelial tumors and their differentiating potential.
J. Neurosurg. 62: 795-805.
21. **Scherer, H.J. (1940):**
Cerebral astrocytomas and their derivatives.
Amer. J. Cancer 40: 159-198.
22. **Schnitzer, J., Franke, W.W. and Schachner, M. (1981):**
Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system.
J. Cell. Biol. 90: 435-447.
23. **Shaw, G., Osborn, M. and Weber, K. (1981):**
An immunofluorescence microscopical study of the neurofilament triplet proteins, vimentin and glial fibrillary acidic protein within adult rat brain.
Eur. J. Cell. Biol. 26: 68-82.

24. **Sharp, G., Osborn, M. and Weber, K. (1982):**
Occurence of two different intermediate filament proteins in the same filament in situ within a human glioma cell line.
Exp. Cell. Res. 144: 385-395.
25. **Trojanowski, J.Q., Lee, V.M.-Y. and Schlaepfer, W.W. (1984):**
An immunohistochemical study of human central and peripheral nervous system tumors, using monoclonal antibodies against neurofilaments and glial filaments.
Hum. Pathol. 15 (3): 248-257.
26. **Wang, E., Cairncross, J.G. and Liem. R.K.H. (1984):**
Identification of glial filament protein and vimentin in the same intermediate filament system in human glioma cells.
Proc. Natl. Acad. Sci. U.S.A. 81: 2102-2106.
27. **Yung, W.K.A., Luna, M. and Borit, A. (1985):**
Vimentin and glial fibrillary acidic protein in human brain tumors.
J. Neuro-Oncol. 3: 35-38.

Summary

During the last seven years immunohistochemistry has become an important tool for the histopathological analysis of neoplasms, including central nervous system tumors.
With immunoperoxidase methods, which can be used on routinely formalin-fixed and paraffin embedded material, in combination with polyclonal antisera, large tumor series have been studied, and the usefulness of this approach has now been well established.
In the central nervous system (CNS), many investigators have reported on the expression of glial fibrillary acidic protein (GFAP), the tissue specific protein constituent of glial intermediate filaments, in normal-, cultured-, and malignant glial cells. Vimentin, the intermediate filament protein normally found in mesenchymal cells, is also expressed by cultured- and immature- glial cells. Tumors usually retain the specific intermediate filament protein of the cell type from which they originate but in addition may also show expression of vimentin.

The present studies were undertaken to document possible changes in class and quantity of intermediate filament proteins in different neuro-ectodermal tumors. Such changes were correlated with histological characteristics such as type and grade of malignancy, and with the micro-environment in the tumor. Using anti-GFAP and anti-vimentin antisera with the peroxidase antiperoxidase method more than 300 human neuro-ectodermal brain tumors were investigated.

In all glial tumors, in the malignant cells, vimentin was expressed in addition to GFAP. Intermediate filament expression appeared to be independent of the grade of malignancy. In all tumors investigated a heterogeneous pattern of expression of GFAP and vimentin was seen. Three possible explanations for this cellular heterogeneity are discussed: a] loss of differentiation (dedifferentiation) in tumor cells derived from a transformed differentiated cell; b] neoplastic transformation of an immature pluripotential cell; c] derivation of the tumor cells from glial cells of different cortical layers. Our findings support the concept that increasing anaplasia (tumor progression) is most probably the result of the dominant proliferation of a more immature and therefore less differentiated cell clone. In various glial tumor types we found enhancement of GFAP expression in neoplastic astrocytes located perivascularly, or invading into leptomeninges or scarr-tissue. These findings suggest that the tumor microenvironment influences the cytoskeleton of the neoplastic cells. Vimentin expression did not show this feature which is in line with the hypothesis that vimentin and GFAP intermediate filament systems are functionally distinct.

In oligodendrogliomas, the expression of GFAP and vimentin by certain tumor cells allowed us to propose two different subclasses, stages of which the intermediate filament expression pattern is analogous to distinct stages in the normal development of oligodendroglial cells. GFAP expression in tumor cells of purely desmoplastic medulloblastomas, especially in the pale reticulin free islands, clearly defined this tumor entity as a differentiating medulloblastoma.

This finding supports the importance of distinguishing this neoplasm from the classical medulloblastoma of the midline which is known to have a much worse prognosis.
It is concluded that intermediate filament protein immunohistochemistry represents not only a significant improvement for the histopathological classification of brain tumors, but is also a valuable tool in the study of the histogenesis and tumor cell biology of these neoplasms.

Samenvatting

Immuunhistochemie is gedurende de laatste zeven jaren een belangrijk hulpmiddel geworden bij de histopathologische analyse van neoplasmata, inclusief de tumoren van het centrale zenuwstelsel.
Met behulp van immunoperoxidase technieken, die gebruikt kunnen worden bij routinematig in formaline gefixeerd en paraffine ingebed materiaal, in combinatie met polyclonale antisera, werden grote tumor series bestudeerd. De bruikbaarheid van deze methoden werd hierdoor bewezen.
Veel onderzoekers beschreven de expressie van glial fibrillary acidic protein (GFAP), het specifieke proteine van glieuze intermediaire filamenten, in zowel normale en neoplastische glieuze cellen, alsmede in glieuze celculturen. Normaliter behouden tumoren het specifieke intermediaire filament proteine van het celtype waaruit zij ontstaan zijn. Soms wordt echter een additionele vimentine expressie bij tumor cellen waargenomen.

Het doel van deze studie was het documenteren van mogelijke veranderingen in aard en hoeveelheid van intermediaire filament proteinen bij verschillende typen neuro-ectodermale hersentumoren. Zulke veranderingen werden gecorreleerd aan histologische kenmerken zoals maligniteitsgraad en micro-omgeving in de tumor. Met behulp van anti-GFAP en anti-vimentine antisera en de peroxidase antiperoxidase techniek werden meer dan 300 neuro-ectodermale hersentumoren onderzocht.

In de tumor cellen van alle astroglieuze tumoren werd naast GFAP- ook vimentine- expressie waargenomen, onafhankelijk van de maligniteitsgraad. Alle onderzochte tumoren toonden een heterogeen patroon van deze GFAP- en vimentine-expressie. Voor dit fenomeen van cellulaire heterogeniteit worden drie mogelijke verklaringen ter discussie gesteld:
a] het verlies van differentiatie (dedifferentiatie) in tumor cellen afkomstig van een getransformeerde gedifferentieerde cel;
b] neoplastische transformatie van een onrijpe pluripotente cel;
c] tumor cellen stammen af van glieuze cellen uit verschillende corticale lagen.
Onze onderzoeksbevindingen steunen het concept dat toenemende anaplasie (tumor progressie) het meest waarschijnlijk voortkomt uit de dominerende proliferatie van een onrijpere en dus minder gedifferentieerde cel-kloon.
In verschillende glieuze tumor-typen werd een versterking waargenomen van GFAP expressie in neoplastische astrocyten welke perivasculair gelocaliseerd zijn, dan wel ingroeien in de leptomeningen of litteken weefsel. Deze bevindingen suggereren dat de tumorale micro-omgeving het cytoskelet van neoplastische cellen beïnvloedt. Het fenomeen werd niet gezien met gebruikmaking van anti-vimentine antisera. De hypothese dat vimentine-en GFAP-intermediaire filament systemen functioneel verschillende entiteiten zijn, wordt hierdoor ondersteund.

De expressie van GFAP en vimentine in oligodendrogliomen leidde tot het voorstel van twee verschillende subklassen, waarbij de expressie van intermediaire filamenten analoog is aan die van de normale ontwikkelingsstadia van de oligodendrocyt.

GFAP expressie in tumor cellen van het pure desmoplastische medulloblastoma, voornamelijk in de reticuline-vrije eilanden, kenmerkt deze tumor entiteit als een differentierend medulloblastoma. Dit onderstreept het belang van een subklassificatie van deze tumor van de groep klassieke medulloblastomen van de "midline", die zoals bekend een veel slechtere prognose hebben.

Concluderend kan gesteld worden dat intermediaire filament proteine immuunhistochemie niet alleen een belangrijke aanwinst voor de histopathologische klassificatie van hersentumoren vormt, maar tevens een waardevol hulpmiddel is bij de bestudering van histogenese en tumorcelbiologie van deze tumoren.

Zusammenfassung

Immunhistochemie wurde während der letzten sieben Jahre zu einem wichtigen Instrument bei der histopathologischen Analyse von Neoplasmata, einschliesslich Tumoren des Zentralen Nervensystems. Mit Hilfe von immunoperoxidasen Techniken, die verwendet werden können bei routinemässig formalinfixiertem und paraffineingebettetem Material, in Kombination mit polyklonalen Antiseren, wurden grosse Tumorserien studiert. Die Brauchbarkeit dieser Methode wurde in dieser Weise bewiesen. Viele Untersucher berichteten über das Vorkommen von gliösem fibrillärem saurem Protein (GFAP), das spezifische Protein der gliösen intermediären Filamente, in sowohl normalen und neoplastischen gliösen Zellen, wie auch in gliösen Zellkulturen. Normalerweise behalten Tumoren den spezifischen intermediären Proteintyp der Zellart von dem sie stammen. Manchmal aber wird eine additionelle Vimentinxpression in Tumorzellen wahrgenommen.

Das Ziel der vorliegenden Studie war die Dokumentation von möglichen Aenderungen in Art und Quantität der intermediären Filamentproteine bei verschiedenen neuroectodermalen Hirntumortypen.
Solche Aenderungen wurden korreliert an histologische Merkmale wie Malignitätsgrad und Mikroumgebung im Tumor. Unter Verwendung von anti-GFAP, anti-Vimentin Antiseren, und von der peroxidasen antiperoxidasen Technik, wurden mehr als 300 neuroektodermale Hirntumoren untersucht.

In den Zellen von allen astrogliösen Tumoren, unabhängig von ihrem Malignitätsgrad wurde nebst einem GFAP auch eine Vimentin-Expression gefunden. Alle untersuchten Tumoren zeigten ein heterogenes Muster von dieser GFAP- und Vimentin- Expression. Drei mögliche Erklärungen dieser zellulären Heterogenität werden diskutiert: a] der Verlust an Differenzierung (Dedifferenzierung) in Tumorzellen die abstammen von einer transformierten dedifferenzierten Zelle; b] neoplastische Transformierung einer unreifen pluripotenten Zelle; c] Tumorzellen stammen ab von gliösen Zellen aus verschiedenen kortikalen Schichten. Unsere Untersuchungsresultate unterstützen das Konzept dass eine zunehmende Anaplasie (Tumorprogression) am wahrscheinlichsten resultiert aus der dominierenden Proliferation eines unreifen und deshalb weniger differenzierten Zellklons.
In unterschiedlichen gliösen Tumortypen wurde eine verstärkte GFAP-Expression gefunden in neoplastischen Astrozyten, die entweder perivaskulär lokalisiert waren, oder einwuchsen in die Leptomeningen oder ins Narbengewebe. Diese Befunde suggerieren, dass die tumorale Mikroumgebung seinen Einfluss auf das Zytoskelett von neoplastischen Zellen hat. Das obenerwähnte Phänomen wurde bei der Verwendung von anti-Vimentin antiseren nicht gesehen. Dies unterstützt die Hypothese, dass Vimentin- und GFAP- intermediäre Filamentsysteme funktionell verschiedene Entitäten sind.

Die Expression von GFAP und Vimentin in Oligodendrogliomen führte zu dem Vorschlag von zwei verschiedenen Subklassen, wobei die intermediäre Filamentexpression der Expression der normalen Entwicklungsstadien des Oligodendrozyten analog ist.

GFAP-Expression in Tumorzellen des rein desmoplastischen Medulloblastomas, vornehmlich in den reticulinfreien Inseln, kennzeichnet diese Tumorentität als ein sich differenzierendes Medulloblastoma. Dies unterstreicht die Unterschiedlichkeit dieses Tumors von der Gruppe der klassischen Medulloblastomen der Mittellinie, die bekanntlich eine viel schlechtere Prognose haben.
Zusammenfassend folgt, dass die intermediäre Filamentproteinimmunhistochemie nicht nur eine Verreicherung der histopathologischen Klassifizierung von Hirntumoren darstellt, sondern gleichzeitig ein sehr wertvolles Hilfsmittel ist zur Erläuterung der Histogenese und Zellbiologie dieser Tumoren.

Résumé

Ces sept dernières années l'immunohistochimie est devenue un instrument important pour l'analyse histopathologique de néoplasmes y compris les tumeurs du système nerveux central.
De grandes séries de tumeurs ont été étudiées à l'aide de techniques d'immunoperoxydase, qui peuvent être appliquées sur du matériel de façon routinière fixé dans le formol et englobé dans la paraffine, en combinaison avec des antisérums polyclonaux. La practicabilité de ces méthodes se trouve ainsi démontrée. Beaucoup de chercheurs ont décrit l'expression de protéine gliofibrillaire acide (GFAP), protéine spécifique des gliofilaments intermédiaires, dans le cadre des gliocellules normales et néoplastiques aussi bien que celui des cultures gliocellulaires. En général les tumeurs conservent la proteine intermédiaire spécifique du type de cellule d'où elles proviennent. Cependant, quelquefois des cellules tumorales montrent une expression additionelle de vimentine.

La présente étude a été entreprise en vue d'établir les changements qui peuvent se produire dans la qualité et la quantité des protéines du filament intermédiaire dans différents types de tumeurs neuro-ectodermales du cerveau. De tels changements ont été mis en rapport avec des caractéristiques histologiques comme le degré de malignité et le micro-environnement dans la tumeur. A l'aide d'antisérums anti-GFAP et anti-vimentine et à l'aide de la technique de peroxydase antiperoxydase, plus de 300 tumeurs neuro-ectodermales du cerveau ont été examinées. Dans les cellules tumorales de toutes les tumeurs astrocytaires il s'observait une expression de vimentine outre celle de GFAP et ceci indépendamment du degré de malignité.
Toutes les tumeurs examinées ont montré un dessin hétérogène de ces expressions de GFAP et de vimentine. Trois explications hypothétiques de ce phénomène de l'hétérogénéité cellulaire sont passées en revue: a] la perte de différentiation (dédifférentiation) dans des cellules tumorales issues d'une cellule différenciée transformée; b] transformation néoplastique d'une cellule pluripotentielle qui n'est pas encore mûre; c] les cellules tumorales descendent de gliocellules situées en différentes chouches corticales.
Nos recherches amènent à des conclusions qui renforcent la conception impliquant que l'anaplasie progressive (la progression tumorale) résulte selon toutes les probabilités de la prolifération dominante d'une clone de cellules pas encore mûres et par conséquent moins différenciées. Dans différents types de gliotumeurs un renforcement de l'expression de GFAP a été localisé dans les astrocytes néoplastiques du côté périvasculaire, ou bien a été observé pénétrant dans les leptomeninges ou le tissu de la cicatrice. Ces conclusions incitent à croire que le micro-environnement tumoral du squelette cytaire influe sur les cellules néoplastiques. Le phénomène a été observé sans avoir recours à des antisérums anti-vimentine. Ainsi l'hypothèse se trouve renforcée qui prétend que les systèmes de filament intermédiaires de GFAP et de vimentine constituent des entités à fonction différencié.

L'expression de GFAP et de vimentine dans des oligodendrogliomens nous amène à la proposition de distinguer désormais deux sous-classes différentes où l'expression des filaments intermédiaires est analogue a celles des phases normales de développement de l'oligodendrocyte.
C'est l'expression de GFAP dans des cellules tumorales du medulloblastome desmoplastique pur, notamment dans les îlots dépourvues de réticuline, qui caractérise cette entité tumorale comme un medulloblastome différencié. Ceci souligne l'importance d'une sous-classification de cette tumeur d'avec le groupe de medulloblastomes classiques du "beau milieu", qui - ainsi que l'on sait - permettent un prognostic beaucoup moins favorable.

En guise de conclusion, nous pouvons affirmer que l'immunohistochimie de la protéine du filament intermédiaire constitue non seulement une acquisition d'importance pour classification histopathologique des tumeurs du cerveau, mais aussi un instrument précieux dans l'étude de l'histogenèse des tumeurs et de la biologie des cellules tumorales.

Dankwoord

De onderzoekswerkzaamheden werden mede mogelijk gemaakt door:
De Nederlandse organisatie voor zuiver-wetenschappelijk onderzoek (ZWO)
en
De Stichting Wetenschappelijk Onderwijs Limburg (SWOL).

Das Genie der Kunst steht in der Rangordnung höher als das der Wissenschaft. Wir würden heute die Fallgesetze kennen, auch ohne Galilei und die Planetengesetze ohne Kepler, Aber wir hätten keine Beethovensche Symphonien ohne Beethoven.

W. Nernst.

Curriculum vitae

De auteur werd op 14 november 1957 in Heerlen geboren, alwaar in 1976 het eindexamen atheneum-B aan de "Scholengemeenschap Sintermeerten" behaald werd.
Van 1976 tot 1982 vond vervolgens de studie geneeskunde plaats aan de Rijksuniversiteit Limburg. Deze werd afgesloten met het artsexamen in juli 1982.
Gedurende de medische studie werd er in het kader van een student assistentschap gedurende tweeëneenhalf jaar klinisch neurophysiologisch onderzoek naar cerebraal geëvoceerde potentialen verricht.
Ondersteund met beurzen van de ZWO en SWOL volgden van 1982 tot maart 1984 neuropathologische research werkzaamheden aan het neurologische instituut van de universiteit van Wenen ("Obersteiner-Institut").
De opleiding tot neurochirurg werd aangevangen in april 1984 met een jaar arts-assistentschap neurologie in het "De Weverziekenhuis" te Heerlen en vanaf april 1985 voortgezet aan de afdeling neurochirurgie van de Rijksuniversiteit te Leiden.